



PHD

Impurity profiling of illicit drugs

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Impurity profiling of illicit drugs

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A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department of Pharmacy and Pharmacology

2016

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Abstract

The pharmaceutical analysis of illicit drugs and the associated impurity profiling can contribute to a harm reduction approach. Impurities in illicit drugs represent a complex problem that requires detailed and reproducible analysis, in part because cutting agents are not often reported by the forensic science community. This research project is focussed on characterising Novel Psychoactive Substances (NPS) and determining their purity/impurity (adulteration) employing a wide range of spectroscopic and chromatographic methods, mainly NMR spectroscopy and Mass Spectrometry. Another aim is to develop a routine, rapid, and quantitative analytical protocol to identify illicit drugs and their impurities (cutting agents) in seized street samples. These two major aims were achieved.

A comprehensive, but critical review of the current literature is provided with respect to the analytical chemistry aspects of illicit drugs and especially their cutting agents. This literature review has a focus on the global concern arising from the current and continuing emergence of NPS and their diverse public health effects. Evidence is provided of illegal drugs, mainly so-called “legal highs” (NPS), from detailed analysis of the contents of amnesty bins and their adulterants provided by the Police from a Bristol night club and from the 2013 Glastonbury music festival, besides other samples they had seized. An accurate chemical assignment of flephedrone regioisomers is made and compared with mephedrone. Impurity profiling of street mephedrone and ketamine samples and their adulterants is presented. A possible link between mephedrone samples is investigated by applying PCA and HCA statistical methods to the ^1H NMR data.

This Thesis is dedicated to my parents

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Abbreviations

µg	microgram
‰	part per thousand
2D	two dimensional
4-FMC	4-fluoromethcathinone
4-MMC	4-methylmethcathinone
ACMD	The Advisory Council on the Misuse of Drugs
BZC	benzethonium chloride
CHLOR	chlorocresol (4-chloro-2-methylphenol)
COSY	correlated spectroscopy
Da	Dalton
DAD	diode array detector
DEPT	distortionless enhancement by polarization transfer
DOSY	diffusion-ordered spectroscopy
EC	external calibration
ECIC	external calibration of the internal solvent signal
EDTA	edetate disodium
EMCDDA	The European Monitoring Centre for Drugs and Drug Addiction
ESI	electrospray ionisation
GC-FID	gas chromatography-flame ionisation detection
H2BC	heteronuclear 2-bond correlation
HMBC	heteronuclear multiple-bond correlation
HOESY	hetero-nuclear Overhauser enhancement spectroscopy
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
Hz	Hertz
IC	internal calibration
IR	infrared
IS	internal standard
<i>J</i>	coupling constant
LC-MS	liquid chromatography-mass spectrometry

m/z	mass over charge
MDMA	3,4-methylenedioxy-methamphetamine
mg	milligram
MHz	mega-Hertz
MS	mass spectrometry
MSG	monosodium glutamate hydrate
MW	relative molecular weight
MXE	methoxetamine
ng	nanogram
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
NPS	new psychoactive substance
<i>NS</i>	number of scans
PCA	principal components analysis
ppm	parts per million
qNMR	quantitative analysis by NMR
QTOF	quadrupole time-of-flight mass spectrometry
<i>S/N</i>	signal-to-noise ratio
SNIF	site-specific natural isotopic fractionation
TSP	trimethylsilylpropanoic acid
UNODC	The United Nations Office on Drugs and Crime
UPLC	ultra-performance liquid chromatography
UV	ultraviolet
VMD	Veterinary Medicines Directorate
v/v	volume by volume
w/v	weight by volume

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Chapter 1

Introduction to impurity profiling of illicit drugs

1.1. New Psychoactive Substances

According to the 2014/15 Crime Survey for England and Wales (CSEW) data, 2.8 million (8.6%) people aged between 16 and 59 had taken illicit drugs in the previous year [1]. Of 3,346 drug-poisoning deaths in England, 2,248 (67%) were linked to drug misuse [2]. Drug legislation in the UK classifies illicit drugs under the Misuse of Drugs Act 1971 into three classes: A, B, and C. These are related to their potential harms and define the maximum penalties for supply, possession, or cultivating drugs. The classification assigned is about probable harm to the user and reflects the possible sentence that might arise for supply or possession of these illicit substances [3].

In recent years, new types of drugs have emerged in the illicit drugs market around the world called New Psychoactive Substances (NPS), “designer drugs” or “legal highs”. Most NPS are not internationally controlled [4], but more than 100 countries (Fig. 1) reported NPS to the United Nations Office on Drugs and Crime (UNODC) [4]. NPS were originally designed to avoid current illicit-drugs legislation by creating compounds with similar chemical structures and therefore similar pharmacological effects to the existing illegal drugs such as cannabis, LSD, and MDMA (ecstasy, E).

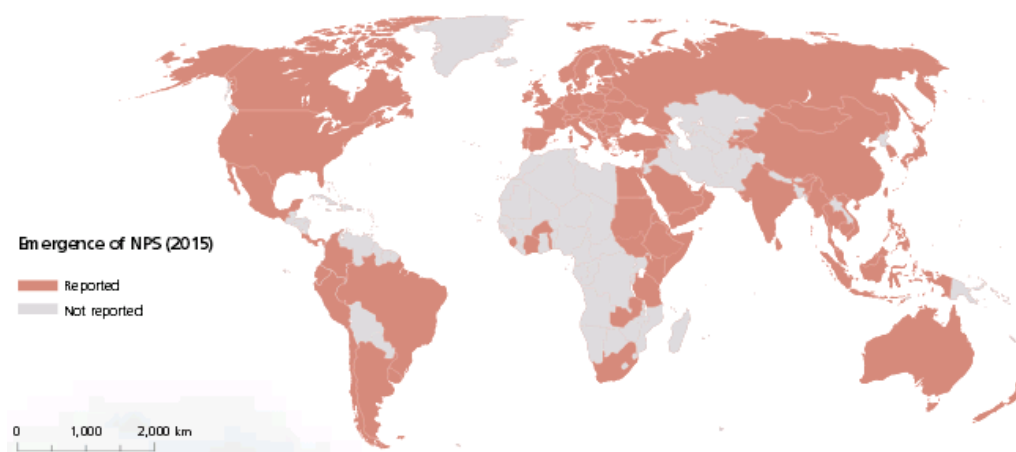


Fig. 1. Global emergence of NPS up to December 2015.

Source: UNODC, Early Warning Advisory on NPS (2015), [5]

NPS are usually sold on the internet under different names, such as “research chemical”, “bath salt” or “legal high” [6, 7]. This global phenomenon of NPS use and supply has brought in to circulation many potent illicit drugs with serious health concerns related to their unknown pharmacology and toxicology. Deaths (60) have been linked to the consumption of NPS in England and Wales in 2013 [8]. The term “New” refers to the availability of the illicit drugs in the black market and not as new inventions. For instance, the synthetic drug ketamine was invented in the early 1960s, but its chemistry was modified recently (e.g. methoxetamine, MXE) to introduce new drugs with similar pharmacological effects to the original one [9]. Cathinone is a main core for the NPS synthetic cathinones which all carry a characteristic β -keto group on the side-chain of the phenethylamines (Fig. 2). This NPS group was first reported occurring in the drugs market around 2005. The earliest reported synthetic cathinone related to MDMA is methylone, 2005, followed by mephedrone (4-methylmeth-cathinone) 2007, and then 3,4-methylenedioxypyrovalerone (MDPV), 2008. Both methylone and mephedrone are among the most used drugs across Europe [10].

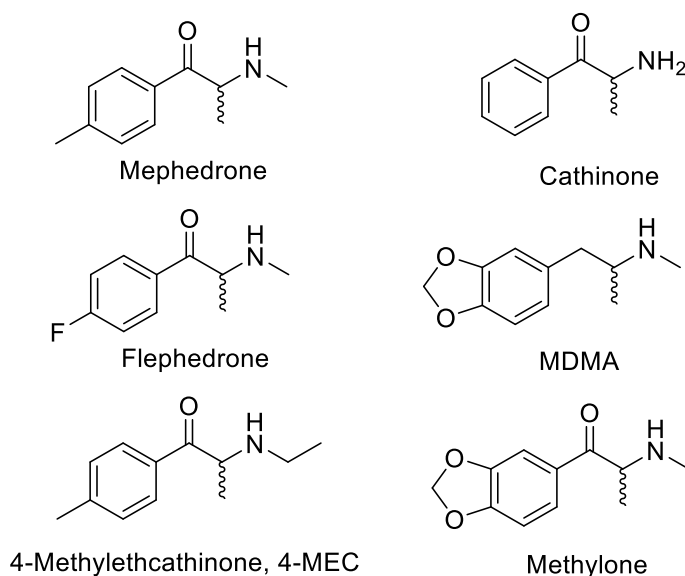


Fig. 2. Synthetic cathinones and their structural similarities to cathinone, together with the cathinone-MDMA derivative methylone

According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 101 new NPS were detected in 2014 besides 349 previously reported, these include: cathinones, cannabinoids, tryptamines, phenethylamines, opioids, benzodiazepines, arylalkylamines, and 13 substances not classified under any group (Fig. 3) [11].

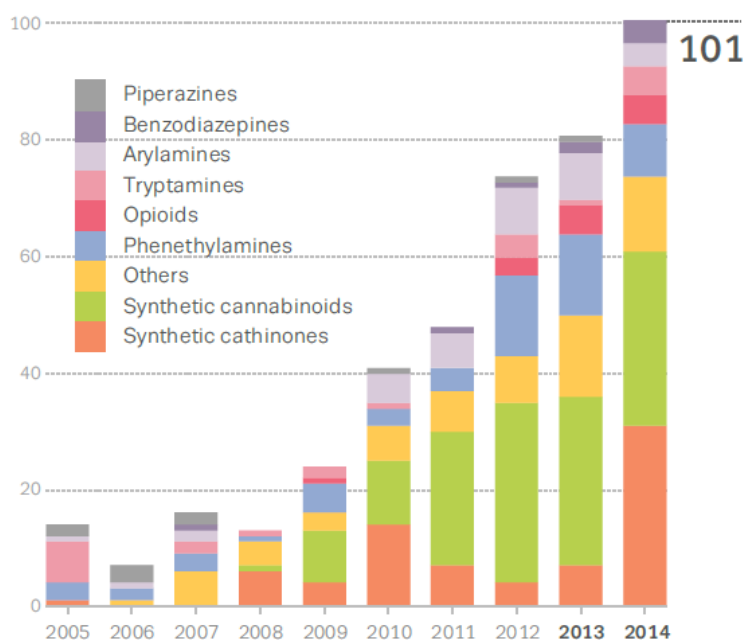


Fig. 3. Number of NPS reported to the EU Early Warning System (EWS) up to 2015.

Source: EMCDDA, NPS in Europe (2015), [11]

The number of NPS was raised to 600 in December 2015 by the Early Warning Advisory (EWA) [4]. The NPS are classified into groups based on their similarity to the existing illicit drugs by the United Nations Office on Drugs and Crime (UNODC) including synthetic cannabinoids, synthetic cathinones, hallucinogens, dissociative anaesthetics, opioids, sedatives besides a group not as yet assigned. These were invented to mimic the effects of the existing drugs, e.g. synthetic cannabinoids as a cannabis alternative, synthetic cathinones have stimulant effects similar to cathinone with similar empathy feeling, and phenylethylamines can show both stimulant, e.g. amphetamines, and hallucinogenic, e.g. 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxy-4-methylamphetamine (DOM) effects [4].

Over the last decade, the market in NPS has been growing very quickly producing challenges to the drugs control systems to monitor these drugs. Among all the NPS, synthetic cathinones and synthetic cannabinoids are the most cause for concern with 1.6 and 1.1 tonnes respectively seized in Europe during 2013 (Fig. 4). Synthetic cathinones form the second largest NPS group monitored by the EMCDDA [11]. The number of new designer cathinones reported during 2014 was 31. This serves to highlight the expansion and the dynamic growth of the NPS market. The explanation of this phenomenon can be linked to different reasons such as more manufacturers, changes in current legislation, and replacement for other drugs [9].

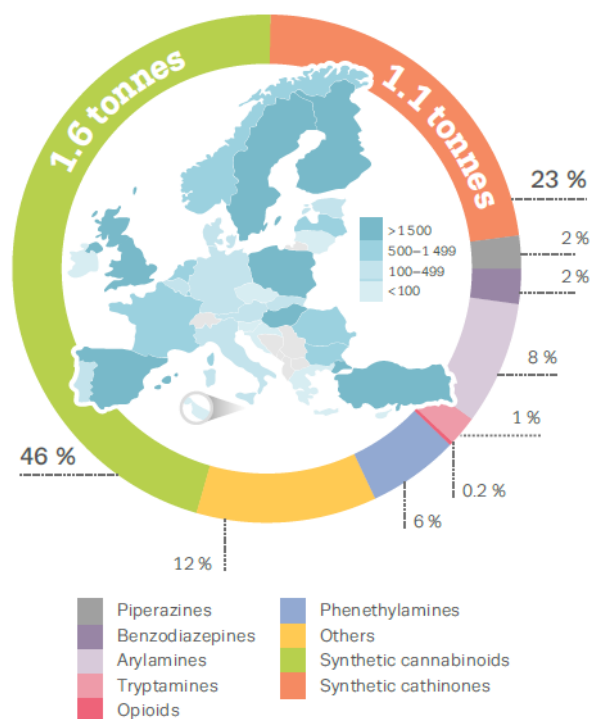


Fig. 4. Number of NPS seizures by country in 2013.

Source: EMCDDA, NPS in Europe (2015), [11]

1.2. Impurity profiling for investigation of NPS

The high similarity between NPS can make the drugs identification challenging [12]. In the illicit drugs journey from the producer to the abuser, the drugs travel through different stages including trafficker, distributor and supplier. At each of these stages, additional substances are commonly added to bulk-out the drugs and make more profit. These substances are known as diluents and adulterants [13]. However, most street drugs contain other impurities beside these ingredients. Impurities are usually due to inexperienced clandestine manufacture, impure starting materials, poor handling and storage conditions. Remaining solvents, synthetic precursors, by-products, degradation and contaminations can provide valuable information relating to a specific route of synthesis. Investigating impurities in illicit drug seizures, by means of identification and quantification, can help to link between different batches and possibly to their clandestine laboratories. Consequently, impurities can be used as a “marker” or “signature” in the impurity profiling investigation [14]. Both chemical and physical profiling of illicit drugs in loose form, tablets and packaging can be sufficient for a forensic investigation to help to tackle organized crime [15, 16].

1.2.1. Adulterants

Adulterants can be defined as any pharmacologically active ingredients added deliberately to enhance the drugs' effect or to facilitate drug administration. For instance, caffeine and procaine are commonly mixed with heroin to help vaporise the drug at a lower temperature [17]. Some adulterants are added by the users themselves during drug administration, e.g. mixing heroin (presumably the free base) with citric acid to make a citrate salt as an injectable formulation [18]. Moreover, drug dealers cut their drugs to mimic the effect of the main drug and/or to mask poor drug quality. Topical anaesthetics such as procaine and lidocaine mimic the numbing effect of cocaine and are used to cut cocaine or might even be sold (to the unwary) as cocaine itself. Cocaine is commonly cut with phenacetin as it is similar to cocaine in its physical properties [18]. Drug dealers also use easily accessible benzocaine as a cutting agent [19]. Paracetamol gives a similar bitter taste to heroin and thus is used to cut heroin [20]. Caffeine is a stimulant frequently used to cut other stimulants, e.g. ecstasy, amphetamine, cocaine [18]. In diverted drugs or abused prescription drugs, the impurities might be preservatives or excipients.

1.2.2. Diluents

Inert substances that are used to bulk out the drugs are called diluents. These usually have a similar physical appearance to the drugs themselves. Sugars are mostly used due to their availability and weight. According to Grabowski's 1984 report [21] in the National Institute on Drug Abuse (NIDA), cocaine is mostly diluted with mannitol, lactose, or inositol [21].

1.3. Harm associated with drug adulterations

The adulterants and impurities discussed above are usually introduced during manufacture, storage or distribution. Typically, drug dealers attempt to cut their drugs with safe and available materials as they want to satisfy their customers and keep their business [22, 23]. The kind and the amount of the cutting agents are usually recommended by drug dealers themselves. However, the safety of these adulterants vary and dangerous adulterants have occasionally been reported, e.g. rat poison, ground

glass [18, 22]. Heroin and cocaine are recounted to be the most adulterated drugs [20]; evidence on their cutting agents, licit use, and health consequences are listed in Table 1.

Table 1

Adulterants reported in street samples of heroin and cocaine together with their licit use and health risk consequences

Drug	Adulterant(s)	Licit use	Potential reason for presence as adulterant	Public health risks	Health consequences
Heroin	Phenobarbital	Barbiturate	Psychoactive drug which facilitates smoking of heroin.	Risk of overdose in IV users who are hypersensitive.	<ul style="list-style-type: none"> • Overdose • Death
	Quinine	Antimalarial medication	Bitter taste similar to heroin and may be used as a diluent. Also mimics the respiratory 'rush' felt by injecting heroin users shortly after administration.	Can cause overdose and a host of other adverse health reactions.	<ul style="list-style-type: none"> • Acute renal failure • Cinchonism • Gastric disturbances • Thrombosis and hypotension (IV use) • CNS overstimulation • Visual disturbances (blindness) • Death
	Clenbuterol	Asthma decongestant and bronchodilator drug ⁴	Reason for inclusion unknown but may have been unintentional contamination.	Can cause overdose and poisoning at moderate to high dosages. Low doses typically cause adverse cardiovascular effects.	<ul style="list-style-type: none"> • Cardiovascular effects • Neuromuscular syndrome • Mydriasis (excessive pupil dilation) • Agitation
	Scopolamine	Anticholinergic alkaloid	Colourless, odourless and tasteless and therefore not easily detectable.	Low doses cause sleepiness and drowsiness. High doses can cause euphoria.	<ul style="list-style-type: none"> • Anticholinergic toxicity • CNS depressant⁴
Cocaine	Lidocaine	Local anaesthetic	Similar, but stronger, anaesthetic effects as cocaine and gives the impression of higher quality cocaine.	Adverse cardiovascular and CNS reactions can occur at low doses. Overdose can occur at excessive doses. Increases the toxicity of cocaine.	<ul style="list-style-type: none"> • CNS problems • Nausea • Vomiting • Dizziness • Tremors • Convulsions
	Hydroxyzine	Sedative, anxiolytic, used as an antihistamine	Unknown, but potentially used in the final processing stages of cocaine manufacturing.	Use in combination with sedative drugs can cause unconsciousness. Rare cases of overdose resulting in CNS problems.	<ul style="list-style-type: none"> • Dizziness • Drowsiness • Gastro-intestinal effects • Tinnitus • Headaches
	Phenacetin	Analgesic substance	Pain relieving properties and similar physical properties to cocaine.	Phenacetin is banned in many countries due to links with renal failure and suspected carcinogenicity.	<ul style="list-style-type: none"> • Analgesic nephropathy • Haemolytic anaemia • Methaemoglobinaemia • Kidney cancer • Bladder cancer
	Levamisole	An anthelmintic medication (used for expelling parasitic worms)	Unknown, however, it is theorised that it gives a more intense high.	Generally no longer used with humans, but still available as a veterinary medicine. Highly toxic.	<ul style="list-style-type: none"> • Fever • Agranulocytosis

Source: ISBN: 978-1-907441-48-6 (pdf), [18]

One of the adverse effects from drug abuse is overdose. This can be as a result of poor quality and purity [18, 24]. When abusers are taking a non-pure (adulterated) drug for the first time, they may think that this is a suitable dose for them and then may suffer an overdose on the next use with a pure sample. Also, users may attempt to exchange their experience on doses [25] and mixing their drugs, which could be dangerous as they can develop tolerance and this will most likely vary between them.

In Canada, the main cause of death among drug addicts is overdose, and that especially among opioids users [26]. Poisonings and deaths have been reported due to the administration of adulterated cocaine, see Table 2 [18, 27, 28, 29]. Adulterants that are added to facilitate specific drug delivery, such as injection or smoking, are typically dangerous. For example, some adulterants are added to facilitate smoking, e.g. caffeine, but users may inject them instead. This can produce serious harm due to the presence of particles or bacteria, and even potentially the presence of other drugs leading to overdose [30]. Infection of users by bacteria, such as *Bacillus* and *Clostridium* species, present in drugs that are not sterile, are common especially among injecting drug users [18].

Table 2

Three adverse health effects caused by consuming adulterated cocaine

Ref	Year	Location	Details	Analysis
[27]	1992	USA	One case of methemoglobinemia due to ingestion of street cocaine adulterated with benzocaine	Urinary analysis identified cocaine and benzocaine
[28]	1982	Dublin, Ireland	Eight cases of strychnine poisoning caused by the inhalation of a substance which was thought to be cocaine	Urinary analysis confirmed the presence of strychnine
[29]	2004	Individual had travelled from Columbia to Rome	Acute intoxication of cocaine adulterated with phenacetin (an analgesic) of a 25 year old male who had 24 packages of cocaine in his digestive tract	Analysis of the cocaine and also urine, blood and gastric content confirmed cocaine with a 30% concentration of phenacetin

Source: ISBN: 978-1-907441-48-6 (pdf), [18]

1.4. Analysis of adulterants contributes to harm reduction

Despite the serious risks associated with the presence of adulterants contributes to in the illicit drugs, these substances are normally neglected during analysis [18, 31]. At minimal amounts adulterants are generally not life-threatening, but they might cause serious health risks at high doses. Analysis of the adulterants present in drugs and identifying their patterns of occurrence could be extremely important to aid workers in hospital emergency departments. Providing treatment to a person who has taken an adulterated drug can be critical and challenging. Medical staff in the Emergency Room should have knowledge and training about the common adulterants used to cut drugs and their possible side-effects if mixed with drugs. Furthermore, monitoring adulterants in drugs can aid law enforcement in their forensic investigation to tackle drug trafficking, distribution, and associated crime [32].

Due to the global impact of the drugs problem, including health costs, especially with the increase in the number of clandestine laboratories and variety of adulterants being used, strategies have been implemented to profile drugs and their impurities. These drug testing strategies have been adopted by several different countries. In 2001, the international drug control leader, UNODC released a manual for characterization/impurity profiling of seized drugs to be used by international law enforcement and drug testing laboratories [13]. One of the most recognized drugs monitoring agencies in Europe is the EMCDDA. This independent scientific research body was founded in 1993 to provide evidence related to illicit drugs and their health consequences, rates of addiction, and monitoring drugs trends across Europe [11].

Since the 1990s, the Drug Information and Monitoring System (DIMS) project in the Netherlands has been active in monitoring drugs and any associated harm related to the content of street drugs. Over 100,000 samples have been handed in to this agency to be analysed, and users were informed if there was any harm associated with their sample [33]. Another international project, the Trans European Drug Information (TEDI) project was launched for drug testing in Europe; Austria, Belgium, Portugal, Spain, Switzerland, and the Netherlands collaborated in this project. The analysis covered the main illicit drugs, besides NPS, between 2008 and 2013 with 45,859 samples analysed. The project findings show a similarity in the drugs market with some interesting differences related to NPS and MDMA [34].

The National Drug Treatment Monitoring System (NDTMS) is a Government Institution to monitor illicit drugs in England based on people receiving treatments for

drug misuse [35]. As a response to the emergence of NPS, the UK Home Office funded the Forensic Early Warning System (FEWS) in 2011. The responsibility of the FEWS is to monitor NPS in the UK in order to inform the Advisory Council on the Misuse of Drugs (ACMD) about any suspicious NPS [36].

Drug abuse in England, Northern Ireland, Scotland, and Wales is also monitored by The United Kingdom (UK) Focal Point on Drugs which is based within Public Health England (PHE). This national partner agency works in parallel with the UK Home Office and supports the EMCDDA by providing regular reports and questionnaires about drugs trends in the UK [37]. The Australian Government has launched the Australian Illicit Drug Intelligence Program (AIDIP) for illicit drugs chemical profiling and monitoring between the Australian Federal Police and the National Measurement Institute of Australia (NMIA). This agency is also collaborating with many other national and international drugs institutes [38].

1.5. Understanding polydrug use

Adulterants in illicit drugs can also be added by the last person in the drug chain, the users themselves. It is common among drug users to mix their drugs during a single drug event to enhance effects or to facilitate drug delivery [39, 40, 41]. This practise is called polydrug use. The risk of drug overdose [42], HIV and Hepatitis C [42], and drug dependence [44] is high among these kind of users. Polydrug users' attitudes can be categorized into three different types:

- i. Mixing two different drugs to enhance the effects [45]. This is common among cannabis and cocaine users by combining these two drugs with alcohol. Another example is a combination of heroin with benzodiazepines or an opioid such as methadone or oxycodone [46]. Ketamine polydrug users commonly use marijuana, alcohol, PCP (angel dust), and amphetamine (speed) [47]. This is possibly to try and reduce the unwanted effects of a drug by sequencing the amount of time between the first and second substance.
- ii. Taking another drug in combination in order to reduce the dependence on other drug gradually. For instance, using cocaine and heroin mixture to balance the withdrawal symptoms of heroin, this is known as "speedball" [44, 47].
- iii. Replace the drug with other drugs due to fluctuations in the price, availability, or regulation. For instance, oxycodone and other opioids are sometimes consumed instead

of heroin. MDMA is commonly replaced with mephedrone or any other available stimulant if the first one is not available [41].

Polydrug practise has been investigated by various studies. A sequencing of polydrug use was reported by Lankenau and Clatts from among 40 ketamine injectors to understand the modes of administering ketamine with other drugs [47]. Another investigation was conducted by the EMCDDA between 2009 and 2010 encompassing 23 countries. Opioid fatalities mostly accrue together with the presence of other substances including alcohol, benzodiazepines, and other opioids [48]. It is important to understand attitudes among polydrug abusers to predict the harm associated with these mixtures.

1.6. Aims and Objectives

Taken together, drug pharmaceutical analysis and impurity profiling contribute to a harm reduction approach. Impurities in illicit drugs represent a complex problem that requires the sharing of knowledge from addiction studies, toxicology, criminology, and the pharmaceutical and forensic analysis of illicit drugs. Furthermore, cutting agents are not often reported by the forensic community. Thus, this analytical chemistry research project is aimed at deciphering the different aspects of impurities currently found in illicit drugs, by gathering information mainly from detailed pharmaceutical analysis of the contents of amnesty bins provided by the police from a Bristol night club and from the 2013 Glastonbury music festival, besides other samples seized by the police. The research is focussed essentially on characterising NPS and determining their purity/impurity (adulteration) employing a wide range of spectroscopic and chromatographic methods namely, 1D and 2D NMR, ESI-MS, EA-IRMS, GC-FID, and UHPLC-MS/MS. Another aim arising from this approach is therefore to develop a routine, rapid, and quantitative analytical protocol to identify illicit drugs and their impurities (cutting agents) in seized street samples.

These two major aims will be achieved through the following six objectives:

1. Carry out a comprehensive, but critical review of the current literature with respect to the analytical chemistry aspects of illicit drugs and especially their cutting agents (chapter 2). This literature review will have a focus on the global concern arising from the current and continuing emergence of NPS and their diverse public health effects.

2. Demonstrate evidence of illegal drugs, mainly so-called “legal highs” (NPS) available in the UK during 2013, by analysing the contents of amnesty bins using NMR spectroscopy as the main technique, but used together with HR-MS (chapter 3). An indicator of current regional drugs trends will be a valuable finding for staff working in hospital emergency departments with regard to the many different drug overdoses or drug-drug interactions with which they have to deal.

3. Provide an accurate chemical assignment of flephedrone regioisomers and compare those with mephedrone by a combination of ^1H , ^{13}C , ^{15}N , and ^{19}F NMR spectroscopies (chapter 4).

4. Impurity profiling, which includes chemical (spectroscopic) and physical (appearance) profiling, of street mephedrone samples and their adulterants using 1D, 2D, and quantitative NMR (qNMR) spectroscopies (chapter 5).

5. Investigate the possible link between mephedrone samples by applying PCA and HCA statistical methods to the ^1H NMR data (chapter 5).

6. Impurity profiling of seized ketamine samples by employing NMR and UPLC-TOF-MS/MS to provide evidence if there is any diversion of ketamine samples from legal sources, i.e. from doctors or veterinary surgeons, into the illicit drugs market in the UK (chapter 6).

A short General Conclusions section will then serve to bring all the major findings together.

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Chapter 2

Literature review: Analytical aspects of illicit drugs

The chemistries and the public health effects of NPS are diverse. The global concern about NPS emergence was discussed recently in the UNODC publication *The challenge of new psychoactive substances* [1]. Their harmful effects, described by the WHO, include: tolerance, dependence, overdose, hospitalisation, risk of Hepatitis C and HIV with injectable drugs, and fatalities. Following seizures of NPS, they are reported frequently to be mixed with other controlled drugs (CDs), e.g. cocaine, methylenedioxymethylamphetamine (MDMA, ecstasy), amphetamine. Considerable health risks are associated with the presence of NPS in mixtures in the absence of any scientific information about their purity and composition. The increasing number of NPS seizures, casework in forensic science laboratories, and fatalities have given rise to the need for a reliable, accurate, and quick analytical methods approach in order to aid law enforcement to prevent abuse by potentially restricting supply [1].

The identification and reporting of NPS as a scientific evidence-base is challenging due to the high chemical similarity between NPS molecules and the broad absence of chemical reference standards, analytical methodologies and refereed literature about NPS analysis. To confirm the identity of unknown substances normally requires synthesis of the drugs and then applying a wide profiling chemical analysis [2]. This approach can be difficult and even impractical when dealing with such a dramatic rise in production as for the illicit drugs market such as NPS. To address this problem, an international collaboration is required to share knowledge and exchange experiences in the analysis and monitoring of NPS. Useful guideline protocols for the qualitative and quantitative illicit drug analysis were recently recommended by UNODC [3], the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) [4], and the European Network of Forensic Sciences Institutes (ENFSI) [5].

Choosing a methodology for illicit drug analysis is based on various criteria including drug type, and availability of suitable instrumentation and reference materials. The basic requirement for the forensic analysis of illicit drugs in seized materials is to provide, to the law enforcement agency, an accurate identity with high confidence under professional practice. According to SWGDRUG, analytical techniques can be placed in three categories: A, B, and C based on their discriminating power (Table 1). Category A (confirmatory techniques) is considered as highly discriminating, with C the lowest.

Table 1

Categories of analytical techniques based on the SWGDRUG recommendations

Category A	Category B	Category C
Infrared Spectroscopy	Capillary Electrophoresis	Colour Tests
Mass Spectrometry	Gas Chromatography	Fluorescence Spectroscopy
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry	Immunoassay
Raman Spectroscopy	Liquid Chromatography	Melting Point
X-Ray Diffractometry	Microcrystalline Tests	Ultraviolet Spectroscopy

Source: SWGDRUG (2011), [4]

Based on the SWGDRUG recommendations for drug analysis, techniques from Category A should provide high professional analysis. Though using this category is required, at least one other supportive technique from any of the three categories is also needed. Using GC/MS is widely recommended and common in practice in illicit drug analysis as this combines two techniques: GC (Category B) and MS (Category A) which addresses the requirements. However, other techniques can be more suitable than GC/MS in analysing specific drug types or forms. For instance, infrared spectroscopy is more suitable as a quick scan device for drugs in salt form possibly providing unique stereoisomer identification [4].

NPS identification using GC-MS requires comparing both the retention time and mass spectrum of the drug with reference materials and these are normally not available [3]. Furthermore, GC analysis requires a volatile and thermally stable analyte otherwise a derivatisation step is needed which can be time consuming [6]. On the other hand, NMR provides unique information related to chemical structure determination and drugs quantification, especially in the case of high similarity between the analytes, such as aromatic ring-substituted amphetamine derivatives, and even in the presence of impurities [3]. The Police often use colour test kits for illicit drugs detection. This kind of analysis is not always reliable especially in the presence of other drugs. For example, Scott Ruybal (2% cobalt thiocyanate ($\text{Co}(\text{SCN})_2$) in aqueous glycerine) is a presumptive colour test used to detect cocaine. However, this test can provide false-positive results especially for drugs in mixtures such as lidocaine and ketamine, and likewise if there are adulterants present [7]. Other procedures should be taken into account when dealing with illicit drug analysis such as sampling and sample preparation. In fact, as a small quantity of material is normally consumed in analysis, this amount should be

representative of the bulk sample. Full details are available in national pharmacopoeias and from international organizations such as UNODC regarding the principle of sampling in drug analysis [8]. With respect to sample preparations, powders should be prepared at 1 mg/mL in methanol. Tablets should be ground into a powder while the contents of capsules must be emptied and treated as for powder preparation procedures.

Drug characterization and impurity profiling of illegal drugs are targeting three important components in seized materials: the main active component, contaminants and adulterants. In the analysis of illicit drugs, additives are known as adulterants or cutting agents while in pharmaceutical preparations they are known as excipients [9]. The fact that most illicit drugs are synthetic, means that contaminants/impurities, by-products, and even solvents can be detected in these samples. Adulterants are another important content that might be present in illegal drugs. It is debatable if these adulterants have been added to enhance the drugs effect, to facilitate administration, or to bulk-up drugs with a cheap adulterant in order to make more profit. It is a powerful tool to use organic impurities and adulterants as a “marker” or “signature” to assist in detecting variations between different seized samples. It is highly likely that different batches of drugs from a single producer are synthesized under the same conditions and, if the same starting materials and solvents are used, they might carry the same impurities [10]. Intelligence investigations can therefore be carried out in this aspect, employing these markers to connect between illicit drugs in different batches and even to their distributor networks and sources of manufacture [11, 12].

From an investigative perspective, chemical and physical drug characterization/impurity profiling can provide valuable information related to: (a) determining a specific link between different seizures, (b) classifying illicit samples from different sources into groups, thus creating distribution patterns to monitor drug trafficking [13, 14], (c) determining the source of the geographical origin of the seizure [15]. Several research projects have been conducted worldwide and in Europe [16, 17, 18], especially in Switzerland [19], where impurity profiling has been successfully conducted in monitoring illegal drug markets and trafficking investigation. One example of the chemical profiling of methylamphetamine, by the National Measurement Institute of Australia (NMIA), is to provide intelligence to the Australian Federal Police that will be strategic in monitoring methylamphetamine. Since the 1970s, sophisticated illicit drugs chemical profiling practises have been developed by the United States Drug Enforcement Administration (US DEA) and the German Bundeskriminalamt (BKA) in order to tackle heroin and cocaine networking and trafficking [11].

2.1. Impurity profiling to determine any specific link between different seizures

In the past, illicit drugs profiling was based on physical characterization only, such as tablet dimensions, mass, colour, and motif [6]. The first attempt to use physical profiling to link between seized tablets was by Gomm et al. in 1976 [6] who compared amphetamine and LSD tablets. However, there are obvious limitations to this method in profiling illicit seized tablets, similar tablets might have different ingredients. Therefore, chemical profiling has emerged as a valuable tool, and is currently used alongside physical profiling for the impurity profiling of illicit drugs [20].

Techniques widely used in the impurity profiling of illicit drugs are Gas Chromatography, such as GC–MS [21, 22], and liquid chromatography techniques, such as High Performance Liquid Chromatography (HPLC) [23]. Indeed, HPLC is normally used to identify drugs and their impurities. Non-destructive techniques such as ^1H and ^{13}C NMR [24, 25], infrared (IR) [25], and ultraviolet (UV) [26] spectroscopies are also used.

One of the first research projects in impurity profiling was reported by Neumann in 1985 in the forensic detection and impurity profiling of opium, morphine and heroin by using GC [26]. Ten years later, the interest in impurity profiling of illicit drugs started as a forensic tool to link between different drug seizures. Signature profiles of alkaloidal impurities and manufacturing by-products present in processed coca leaves were determined using chromatographic methods [27]. This study included a wide variety of chromatography techniques using packed- and capillary-column GC and GC with flame-ionization, electron-capture, nitrogen-phosphorous, and mass spectrometric detection, and HPLC-UV [27].

An early illicit drug content and purity investigation was reported by King in 1994 [28] where GC was used to test more than 4000 samples. These were provided by the Police and Customs and had been seized over 4 years. This impurity profiling investigation showed that almost all the amphetamine samples were imported from the Netherlands during 1993 [28].

In a following study [9], King examined the purity of illegal drugs in the UK and related to concerning when they were seized and the geographical region of the seizure. HPLC was used for quantitative analysis and these data were combined with the intelligence database of the Forensic Science Service (FSS). A comparison has been made between imported illegal drugs (i.e. Customs seizures of imports) and street drugs (i.e. Police seizures of drugs being dealt) regarding their purity and adulterations [9].

Abuse of amphetamine-type substances (ATS), e.g. amphetamine, methylamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethylamphetamine (MDMA), is reported to be higher than both cocaine and heroin abuse added together [29]. In the case of synthetic drugs, contaminants, e.g. by-products, intermediates and impurities, can facilitate the identification of the synthetic route and sources of precursors. This may link to the location of manufacture of these illicit drugs. Other possible sources of impurity might be from packaging, poor handling, and/or degradation during storage [30].

Comprehensive studies have been done on the profiling of illicit MDMA tablets to determine their sources and link between different seizures [10, 30, 31, 32]. Cheng et al. examined the contents of MDMA tablets from 89 different seizures in Hong Kong between 2002 and 2004. The impurity profiling was carried out using GC-MS after extracting the tablets in diethyl ether under alkaline conditions. A total of 19 impurities were identified and used as a fingerprint to link between these different tablets. Cutting agents were examined as well, e.g. caffeine. The tablets were classified into different groups by Hierarchical Cluster Analysis (HCA) showing the capacity of this approach to discriminate between these seizures based on their sources [33].

Determining a specific synthesis route to MDMA by examining the impurities present in precursors and intermediates used, e.g. sassafras oil, safrole, isosafrole, piperonal, β -nitroisosafrole, piperonylmethylketone (PMK) was achieved by Gimeno et al. [32]. The target compound, MDMA, was synthesised using isosafrole via reductive amination and impurities were monitored by MS. The results show the possibility of discriminating two main routes for PMK and MDMA samples by detecting traces of isosafrole glycol and β -nitroisosafrole in the final product.

Research by Bora et al. [34] investigated the possible links between MDMA tablets by measuring elements present in the seized tablets using inductively coupled plasma optical emission spectrometry (ICP-OES). A total of 248 seized MDMA tablets were subjected to ICP-OES analysis to measure 11 elements including: Mg, Zn, Ca, Al, K, B, Na, Ba, Fe, Cu, and Pt after each sample was dissolved in nitric acid and heated in a microwave oven. The results confirmed the presence of some elements that are used in MDMA tablet production such as Mg, Pt, and B. Residual boron is most likely derived from borohydride which is used as a reducing agent, while Pt and Mg were potentially from PtO₂ (catalyst) and magnesium stearate (tableting agent), respectively. It was concluded from the data that the presence of a specific metal might link to a

specific synthetic route. This approach can be used to link between different tablets, together with the physical properties of the tablets [34].

In a separate study, Infante et al. [35] investigated metal contaminants derived from adulterants in illicit samples of heroin in southern Spain. The contents of 198 heroin samples were analysed focusing on 6 elements, Mn, Ca, Cu, Cd, Fe, and Zn. 93.4% of the samples contained a high concentration of calcium. This was mostly from excipients containing salts of this metal, e.g. calcium bicarbonate. All the other listed elements are usually present in metal containers, where they have the potential to be introduced during morphine extraction from opium poppy, causing toxicity concerns especially in heavy users of heroin.

Detailed analytical reference data on using three synthetic routes to MDMA were reported by Renton et al. [25]. Analytical chromatographic data of precursors, i.e. safrole and isosafrole, intermediates, namely: isosafrole glycol, *N*-formyl-3,4-methylenedioxy-amphetamine, PMK, *N*-formyl-3,4-methylenedioxymethyl-amphetamine, and 1-(3,4-methylenedioxyphenyl)-2-bromopropane, by-products, and the final product MDMA were obtained by thin-layer chromatography (tlc) and GC-MS. Additional chemical structure confirmation data were determined by ¹H- and ¹³C NMR, IR, and UV spectroscopies, and X-ray diffraction. These data were used as a reference to monitor the synthetic route to MDMA by tracing the starting materials, intermediates and by-products in seized MDMA. In a separate study by Bohn et al. [24], twelve impurities were characterized and used as “synthesis markers” to identify the synthetic route to MDMA. They employed tlc, ¹H NMR, and MS for the analysis of these impurities. Similar procedures were used [30, 36] to link between MDMA tablets based on their impurity signature and, moreover, to identify their synthetic routes.

A total synthetic method and impurity profiling of mephedrone were developed by Santali et al. [37]. In this method, mephedrone was prepared in both HCl and HBr salt forms. The first chromatographic separation method was reported in this study to quantify mephedrone and possible adulterants; mephedrone characterization was supported by GC-MS, HPLC, NMR, IR, and UV data [37].

2.2. Adulterants and classifying illicit samples from different sources into groups

Information related to illicit drug adulterants is usually neglected in toxicology or forensic laboratories, focusing only on illegal drugs identification and quantification, as these aspects are used by the judiciary [38]. Only really a few literature reports are

focused on adulterants except for those “high volume” drugs [39], e.g. heroin [40], cocaine [41], and amphetamine-type stimulants [16]. However, most investigations of adulteration are after fatality [42] or for use in judicial purposes. One example of this is heroin cut with scopolamine, a medication used in the treatment of motion sickness, nausea and vomiting, which spread in New York City causing severe anticholinergic toxicity [42].

In their comprehensive review on adulterants in illicit drugs and their toxicity, Cole et al. [38] classified the reasons behind such adulterants into four causes: to add bulk, to enhance or mimic biological effects, to facilitate administration, and as contaminants from poor manufacturing conditions. Their review concludes that most common cutting agents used to cut drugs are legal, cheap, and available, e.g. caffeine, procaine, paracetamol, sugar (glucose, sucrose, mannitol). Their findings confirm that drug dealers mostly cut drugs with benign substances, e.g. caffeine, sucrose. Other adulterants are reported to be toxic at high doses and this is especially relevant in the case of injectable drugs [38].

HPLC-DAD was developed and validated to determine cocaine and its adulterants in seized street samples in Italy. In this method, the authors identified cocaine, caffeine, lidocaine, procaine, phenacetin, and levamisole using a C-18 column with potassium phosphate buffer-acetonitrile mobile phase under isocratic conditions [43]. The method was introduced as a robust and rapid method for the routine analysis of cocaine and any impurities over the ranges 0.01-0.50 mg/mL with Limit of Detection (LOD) 0.004 mg/mL and Limit of Quantification (LOQ) 0.01 mg/mL. The data were cross validated with previous results obtained by the GC-FID method reported by Schneider and Meys in 2011 [44].

Variation in illicit drugs trends can be tested by monitoring sedatives in seized samples. Changes in contents of adulterants was detected between 1981 and 1992 in 383 street samples of heroin when they were tested in a study conducted by Kaa in 1994 in the western part of Denmark [45]. The purity of heroin samples was compared based on their origin and year of seizure. Both heroin hydrochloride and heroin free base were gaining same popularity in the 1980s. However, the free base form of heroin has become popular recently. From the mid-eighties, heroin samples from Asia were discovered containing noscapine, predominantly at high concentrations, whereas caffeine, procaine, and sugar were more common adulterants at the beginning of the eighties. Later in the eighties, heroin samples were cut with phenobarbital and methaqualone, and this practice then decreased to be replaced with adulteration with a

combination of paracetamol and caffeine during the early nineties. The heroin samples were reanalysed to check the stability of these samples. This heroin/adulterants trend could be extremely valuable to monitor changes in the market which then might also possibly link to the sources of manufacture of these cut samples. Another long analytical study by Broseus et al. [18] investigated frequent adulterants of cocaine and heroin samples confiscated between 2006 and 2014 in Switzerland. The study involved more than 6000 cocaine and 3000 heroin samples showing quite stable small variations in the combination of heroin adulterants compared with more dynamic changes in the cocaine samples. In terms of number of adulterants, cocaine samples were cut with phenacetin, levamisole, lidocaine, and caffeine, while heroin samples were found to be adulterated with only two cutting agents, caffeine and paracetamol.

Lapachinske et al. [14] developed a method to monitor international trafficking of cocaine by determining the cocaine purity in Brazil. The method employed gas chromatography with nitrogen phosphorous detection (GC-NPD) to quantify cocaine and its adulterants in the seized samples. The cocaine samples were found to be laced with caffeine, 4-dimethylaminoantipyrine, levamisole, lidocaine, and phenacetin. The data show that cocaine intended for international trafficking was impure before leaving Brazil with purity ranging from 16.5% to 91.4%. This important finding changed the belief that most illicit drugs are cut by distributors. It is in agreement with a previous detailed investigation by Coomber, reported in 1999, about the nature of heroin adulterants in use in the US in the 1990s [40]. Changes in adulteration practices can therefore be used to monitor international illegal drugs trafficking.

Lurie et al. [46] have developed a sensitive, rapid, and reproducible chromatographic method for impurity profiling of heroin and its adulterants using ultra-high-performance liquid chromatography (UHPLC). The following compounds: morphine, acetylcodeine, codeine, *O*3-monoacetylmorphine, *O*6-monoacetylmorphine, noscapine, and papaverine were successfully separated on reversed-phase (RP) chromatography using a 1.7 μ m Acquity UPLC BEH C18 (2.1 mm x 150 mm) column with a phosphate buffer-acetonitrile mobile phase. The separation was achieved within 20 min. A different column was used, a 1.7 μ m Acquity CSH Fluoro-Phenyl (2.1 mm x 150 mm), where adulterants interfered with the target compound heroin. This method was able to detect specific impurities within the heroin samples as low as 0.02% [46].

Illicit drugs can also be cut with other psychoactive substances to enhance or to reverse the unwanted effects of an ingested drug. MDMA tablets, sold in the UK, were

examined by Sherlock et al. [47]. The tablets were grouped based on their physical appearance and analysed by GC-MS. Some tablets contained no MDMA and others were shown to contain various types of psychoactive substances, e.g. amphetamine, methylamphetamine, and ketamine. Two ketamine tablets were found to contain 186 mg and 197 mg respectively, which is equal to about 2.5 mg ketamine/kg body weight. If the ketamine is administered orally, then only one sixth of this amount reaches the body's circulation [48]. A report suggests that analgesia is produced by oral doses of 0.5 mg/kg [49]. Therefore, in this case, there is a high risk of toxicity if a user consumes more than two tablets thinking they may contain MDMA. Other adulterants detected in MDMA tablets were caffeine and paracetamol. As a mark of illicit drug manufacturing practice, the concentration of MDMA between the tablets varied from 0 to 160 mg even for tablets sold under the same brand name. This variation can produce considerable toxicity and therefore harm.

Recently (late 2015), Gama et al. [50] reported methodology for routine illicit drug analysis using No-deuterium proton NMR (No-D NMR). The method was tested on 71 cocaine samples, one MDMA and one of methylone seized by the Police from different areas in Brazil from January 2012. The LOD value of cocaine in this method was ≈ 2 mg/mL. Cutting agents, namely lidocaine, caffeine, and phenacetin, were well resolved from cocaine signals. Sample purity and adulterants were used to map the samples based on their cutting agents. This method was introduced as a non-destructive, inexpensive and fast technique compared to GC [50].

Full ^1H NMR assignment and two-dimensional diffusion-ordered spectroscopy (2D DOSY) ^1H NMR of 24 seized heroin samples was carried out by Balayssac et al. [51]. Other 2D NMR experiments were applied to allow unambiguous assignment, e.g. homonuclear correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple-bond correlation spectroscopy (HMBC). The study provides clear characterization of heroin samples in the presence of related impurities, e.g. 6-acetylmorphine, acetylcodeine, morphine, noscapine, and papaverine. Also, this analysis was tested in the presence of adulterants, e.g. caffeine and paracetamol, in nearly all samples as well as lactose, lidocaine, mannitol, and piracetam.

Table 2

Illicit drugs adulteration summary and their associated analytical techniques

Drug	Technique	Adulterant	Sources/Uses	Ref
MDMA tablets	GC-FID, GC-MS	caffeine, ephedrine, paracetamol, procaine, lignocaine, amphetamine, phenylethylamine		[9], [33], [47]
	ICP-MS	Mg	magnesium stearate (tableting agent)	[34]
		Pt	from PtO ₂ used as a catalyst	
Heroin		paracetamol	- increases the volatility of heroin freebase	[41]
		procaine	- to relieve the pain of an i.v. injection	
		phenobarbital, methaqualone, paracetamol, caffeine		[45]
Cocaine	No-D NMR	lidocaine, caffeine, phenacetin		[50]
	HPLC-DAD	caffeine, lidocaine, procaine, phenacetin, levamisole		[43]

To test the applicability of quantitative NMR (qNMR) method in this area of illicit drugs analysis, heroin, its impurities and adulterants were assayed by qNMR. The data analysis shows good agreement with GC for quantification. This study therefore shows the viability of qNMR to establish a signature profiling of illicit drugs [51]. The NMR method also allows the simultaneous detection of drugs in mixtures with impurities and adulterants using a single screening technique of high fidelity. Another non-destructive method to determine illicit drugs with their adulterants was reported by Rodrigues et al. in 2013 [52]. They combined both attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) and principal component analysis (PCA). The study was designed to build a statistical model using the ATR-FTIR data to classify cocaine samples based on their purity/impurity contents. The model split the samples into three principal components. The first component discriminated between the adulterated sample with lidocaine, caffeine, and benzocaine. The second, PCA2, was classified between cocaine free base and cocaine salt. Both cocaine and its cutting agents were quantified as well using GC-MS. These kind of classification models are valuable tools for a rapid non-destructive method to support criminal investigators.

There is no evidence in the literature related to the impurity profiling of ketamine and its adulterants. Cole et al. found [38] that both ketamine and GHB have little in the literature about their adulteration and that they are mostly diverted from legal sources [53, 54]. However, as set out above, ketamine is used as an adulterant for other drugs such as MDMA [47]. A summary of the adulterants used to cut illicit drugs, illicit use, and their detection techniques is presented in Table 2.

2.3. Isotopic analysis for identifying the origin of drug samples

In recent years, the clandestine manufacture of illegal drugs has developed and many different illegal drugs are now available in high quality grades. This means that using impurity profiling to obtain intelligence information to aid law enforcement has become correspondingly less useful [55]. The use of isotope ratio analysis for drug profiling is increasingly appreciated by the forensic science community in cases where drugs are more pure and so less impurity is present in the seized samples [56-60]. Isotope profiling can be used as a powerful technique in this case for forensic investigation to link between seized samples and so possibly to identify their origin of manufacture. Measurements of stable-isotope ratios (δ) have been used to track back to the geographical origin of species in other fields such as migrating butterflies [61]. This

technique has also been used in other non-biological materials applications, e.g. to determine emerald trade routes [62] and environmental issues [63]. The application of this technique has recently been used to identify the origins of natural and synthetic illicit drugs [64].

2.3.1. Naturally occurring illicit drugs

In naturally occurring substances, stable-isotope ratios of $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\delta^{32}\text{S}$ are effected directly by their growth environments, e.g. soil and climate. These variable factors are referred to as geo-location effects [65]. They have been used to link back to their geographical origins certain naturally occurring illicit drugs, e.g. cannabis, cocaine, morphine (for heroin) [66].

Cocaine samples were linked successfully (96%) to their geographical regions by using their carbon and nitrogen isotope ratios in a study by Ehleringer et al. in South America [56]. A combined model of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope-ratio alkaloid traces was used to classify the cocaine samples. Other alkaloids in the samples were successfully employed for more discrimination between the samples, e.g. truxilline was correlated positively with cocaine $\delta^{15}\text{N}$, while a negative correlation was observed between cocaine $\delta^{13}\text{C}$ and trimethoxycocaine. This strategy can be used to trace the origin of cocaine samples and potentially to monitor their trafficking routes.

Morphine (for heroin) is usually harvested in different regions in the world where soil and humidity are suitable for its growth. Most heroin comes from Asia, Colombia, or Mexico. To differentiate between the sources, isotope ratio analysis was used to measure the values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ [67]. Casale et al. [68] examined the sources of 20 samples from different packages in HCl form seized from the North Korean-flagged cargo vessel, Pong Su and examined by both the Australian and the US authorities in 2003. Impurity and manufacturing by-products were previously tested. The chromatographic profile signature of these samples showed unusual impurities which is consistent with a new processing method or even a new region of production. The isotope ratios were measured for the heroin samples and compared with over 200 authentic heroin samples obtained from known sources: Southeast Asian, Southwest Asia, Mexico, and South America. Elemental analysis-isotope ratio mass spectrometry (EA-IRMS) and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) techniques were used to measure $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ respectively [68]. The origin of the

heroin samples did not match any of the known origin isotopic signatures and this therefore suggests a new source of heroin samples.

2.3.2. *Synthetic illicit drugs*

Although stable isotope ratio analysis has been applied successfully to determine the origin for such natural product drugs as cocaine and morphine, this analysis can also be applied to synthetic drugs. This analysis is carried out on their natural precursors [69, 70]. For example, MDMA is a synthetic drug, but natural product starting materials are used in its synthesis. They can be traced using their isotope ratio, e.g. safrole [71]. Furthermore, the variations in the natural abundance of the isotopes can be used to discriminate between different seizures. The ability of the stable-isotope ratio measurement to uncover any possible link between different synthetic drugs was tested by Collins et al. in their study investigating a number of cathinones [55]. Most cathinones share the same synthetic method, starting with a β -ketoarylalkane followed by bromination α to the keto group, and finishing with an amination step. Isotope ratios of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^2\text{H}$ vary from one seizure to another. These variations were used to classify the seized cathinone samples into groups [55] in part depending upon the synthetic routes used, mainly a substitution reaction of the α -bromoketone to afford the substituted amine.

Further analysis can be done by using this variation in the isotope values technique to determine a specific synthetic route [60, 67, 72]. A comprehensive review was recently presented by Collins and Salouros on measuring the stable isotope ratios of carbon, hydrogen, and nitrogen in methylamphetamine to identify the origin of the starting materials [73]. This was successfully achieved by measuring the isotope ratios of the precursors ephedrine and pseudoephedrine for samples synthesised from known sources. These data were then compared to the isotope ratios of more than 1600 seized methylamphetamine samples to establish a link between them and their synthetic precursors [73]. The samples could successfully be linked to their precursors; the authors believe this to be a powerful technique that can be used for a routine analysis of cathinone profiling, assessing the variations between seized samples in order to find any possible linkages.

Buchanan et al. [60] provide evidence that, in the synthesis of MDMA using Pt/H_2 reductive amination, the $\delta^2\text{H}$ values of MDMA-HCl are sensitive to the length of imine stir time. Moreover, the $\delta^{15}\text{N}$ values can be changed based on the molar excess of

methylaniline used during the reaction. These variations can therefore be measured to differentiate between batches according to the specific methods used along their synthetic route and hence aid in identifying seized batches prior to cutting (adulteration) and supply.

2.4. General conclusions

Generally, different strategies have been used for impurity profiling of illicit drugs in intelligence investigations. One of these strategies is to identify the illegal drug source by combining purity and adulteration analysis findings with the intelligence database. While another links the presence of specific impurities as “markers”, e.g. synthetic precursors and chemical intermediates, to a particular synthetic route for a possible link to their source and potentially to their manufacture. Further approach is to classify the seized illicit drugs into groups based on variations in their impurities using statistical data analysis, e.g. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Determining the origin of illegal drugs in seized materials has been successfully achieved for both naturally occurring and synthetic illicit drugs. Other analytical studies have identified adulteration as their focus in order to help to understand the reason for using and the harm associated with these introduced substances. Long-term studies monitoring the frequent adulterants in seized heroin and cocaine have also been undertaken.

A wide range of chromatographic and spectroscopic methods have been reported for the investigation and impurity profiling of illicit drugs. After critically reviewing the literature, the most frequently used analytical techniques for illicit drugs impurity profiling are gas and liquid chromatography, e.g. GC-MS, GC-FID, and LC hyphenated with MS or with UV diode-array detection. In fact, as most illicit drugs are in salt form, when using GC it is required to form a volatile drug free base or carry out a derivatization step. For the LC approach, a MS detector is useful or pure reference materials are needed. Another difficulty related to MS detection is that some highly similar NPS can have the same molecular mass.

Metal contaminants have been investigated in seized heroin and in MDMA tablets using ICP-OES. Only a few studies have been reported using 1D and 2D NMR spectroscopic techniques for impurity profiling. Other non-destructive spectroscopic methods to determine illicit drugs with their adulterants are ATR-FTIR and handheld Raman spectrometers. Possible links between seized samples and their geographical

origin have been investigated by measuring the isotope ratio of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^2\text{H}$ using isotope ratio (IR)-MS.

Illicit drugs chemical profiling is not a new innovation; a few agencies worldwide have adopted this strategy for specific drugs, e.g. cocaine, heroin, methylamphetamine. In practice, more efforts should be made especially with NPS in order to monitor national and international illicit drugs trafficking. Impurity profiling of illicit drugs contributes to harm reduction procedures. It is also important for policy makers, emergency departments, addiction treatment providers, and forensic investigations to aid law enforcement. At this time, the reporting of impurities such as adulterants is not a common practice in illicit drugs analysis, especially for NPS.

To fill this gap, these research studies will therefore investigate the impurity profiling of NPS such as mephedrone, flephedrone, and ketamine and aim to shine a spotlight on any new emergence of toxic drugs, e.g. dimethyltryptamine (DMT) and the 2C-X family. As stated above, the most challenging part of accurate and reproducible NPS analysis is the absence of reference standard materials and the application of analytical techniques that provide accurate chemical assignments of the drugs. Most NPS are similar in chemical structure or they occur within mixtures with other drugs or adulterants. In this case, NMR spectroscopy can give unique patterns for each drug and its impurities. This investigation will explore the advantages of NMR as a powerful technique in drug characterization and impurity profiling. Findings from these studies will be used to: provide accurate drug assignments in the presence of their impurities (flephedrone, chapter 4), classify illicit samples into groups based on their impurities (mephedrone, chapter 5, and ketamine, chapter 6), determine the source including geographical origin of the seizure (ketamine, chapter 6), and obtain distribution patterns for some areas in the UK, then being able to provide these data to the Police (amnesty bins, chapter 3). Thus, the laboratory-based pharmaceutical research aspects begin with detailed analysis of amnesty bin contents and other seized samples from the South West of the UK supplied by the Avon and Somerset Constabulary.

2.5. References

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Chapter 3

Analysis of amnesty bins as an indicator of current drugs trends

3.1. Introduction

In the UK, drug users represent 8.9% of the population (2011-2012 data) [1], and the widespread use of drugs is estimated to cost society £15.4 bn annually [2]. The internet plays an important role in the emerging drugs “marketplace” especially for NPS, sometimes referred to as “legal highs”. Responding to this phenomenon, the Forensic Early Warning System (FEWS) was established and funded by the UK Home Office in 2011 [3]. Monitoring illicit drugs, i.e. monitoring the change in their availability over time, can improve our understanding of the use of these substances. Profiling NPS availability in the European Union (EU) has been monitored by “Internet snapshot surveys”, a study conducted by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [4]. Understanding regional drug use and trends is a valuable finding to staff in hospital emergency departments who have to be able to deal with many different drug toxicity situations [5].

Analysis of urine in wastewater to identify regional variations in recreational drugs use has recently been reported [6, 7]. Estimates of drugs consumption can be made by measuring the amount of illicit drugs and their metabolites in urine [8, 9]. This approach might be limited by the stability of drugs and their metabolites in sewage and taking into account other factors that might affect the analysis, e.g. drug concentration, impact of bacterial metabolism [10]. A questionnaire survey was employed as well for drug monitoring [11]. However, surveys are limited, and subject to various confounding factors [12].

Using an amnesty bin to track current drug consumption has been recently reported in other independent studies [13-15]. This method was first introduced by Ramsey and others as a new method for monitoring illicit drugs use in a dance venue [13]. These amnesty bins are usually located at night club and music festival entrances; people can discard their drugs without any comeback. Therefore, pharmaceutical analysis of amnesty bins can provide reliable sources of data by yielding information about the drugs’ physical description (crystalline, liquid, powder, tablet, and herbal), method of administration (a salt being typically water

soluble and so a solution can be injected, or a freebase being sufficiently volatile on heating to smoke), and packaging systems (plastic bag, folded paper, tablets with a logo). Further analysis may lead to identifying manufacturing methods by detecting by-products, solvent residues, and especially impurities. This methodology could also be used to monitor purity, adulterants, and production batches for possible links between different seizures for forensic investigation.

For detecting unknown drugs in seized materials, GC-MS and LC-MS are both employed. Both methods require reference materials [16, 17]. Colour tests, e.g. the Marquis reagent, are mostly used by the Police [18]. However, such a test can give false negative results in the presence of adulterants or on changing the pH of the water that was used to dissolve the illicit samples [19]. Recently, many analytical methods have been developed and introduced to the Police as quick, non-destructive, and reliable techniques, e.g. hand-held Raman, benchtop FT-IR, and benchtop NMR [20, 21]. However, all of these methods are presumptive tests and the results need additional methods to be finally confirmed [16]. High-resolution magic angle spinning (HR-MAS) NMR spectroscopy was used successfully to identify illicit drugs in seized tablets and blotter papers [22]. Using NMR for illicit drugs analysis in seized materials is listed as a recommended technique under Category A by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) [23].

The purpose of this chapter is to determine what illicit drugs are currently available, to provide a quick snapshot to aid law enforcement focusing on NPS, and to corroborate the data with other regional drugs analysis. The main analytical technique that will be used in the analysis of the contents of amnesty bins is ^1H and ^{13}C NMR spectroscopy supported by electrospray ionisation-mass spectrometry (ESI-MS) and ATR-FTIR spectroscopic analysis. 2D NMR (HMBC, HSQC, DEPT, ^{15}N HMBC, ^{19}F , H2BC) spectroscopic analysis is run in some cases to provide additional analytical data. The research in this chapter will show the viability of using NMR spectroscopy for the analysis of illicit drugs on a large scale, i.e. in large numbers such as from amnesty bins, and especially with minimal sample preparation. Further investigations will then be applied to drug purity and adulterants. Impurity profiling of illicit drugs will provide a better understanding of the harm associated with impurities in illicit drugs.

3.2. Experimental

3.2.1. Materials

All solvents (HPLC grade 99%), deuterium oxide (99.8 atom % D), dimethyl sulfoxide ACS reagent, $\geq 99.9\%$, mephedrone hydrochloride salt; ketamine hydrochloride, creatine monohydrate, and monosodium glutamate were purchased from Sigma-Aldrich (UK). Deuterated methanol (99.8 atom % D) was purchased from Cambridge Isotope Laboratories (USA). Reference standards for flephedrone hydrochloride salt were purchased from LGC Standards (UK) and for benzocaine B.P. from J. M. Loveridge plc (Southampton, UK). A pure sample of MDMA from the amnesty bin was recrystallized, analysed by NMR, and used as a reference material. Ten sealed evidence bags from the Glastonbury music festival (2013) and one from a Bristol night club were obtained from the Drug Expert Action Team (DEAT), Avon and Somerset Constabulary, UK. The illicit drugs were contained in glass bottles, plastic containers, and samples as crystals and powders in plastic bags, capsules, and tablets, besides cannabis grinders, cannabis, and other herbal materials.

3.2.2. Instrumentation

All measurements were run at 20-25 °C on Bruker 400 and 500 MHz NMR spectrometers, and Electrospray Ionisation Mass Spectrometry (ESI-MS) on a Bruker Daltonics micrOTOF. ATR-FTIR spectroscopic analysis was performed on a Bruker FT-IR spectrometer (Bruker Optics, Billerica, MA, USA) equipped with the TICTAC drugs identification database. The data were processed using OPUS software.

3.2.3. Analysis protocol

The Police provided 207 tablet and powder samples from an amnesty bin in a Bristol (UK) nightclub (2013) plus 279 samples from the Glastonbury music festival (2013) in addition to ~62 herbal samples that were not analysed further. Substances were received within a big evidence bag containing small bags which were each sorted separately. Tablets with the same colour and logo within the same bag were

labelled with the same code. One tablet from each batch was then analysed. MDMA tablets were ground, dissolved (50 mg/mL), vortexed for 1 min and centrifuged (3 min), then an aliquot submitted to ^1H NMR analysis. The samples identities were also confirmed by ESI-MS. All MDMA tablets were scanned and identified correctly from the TICTAC Library using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy. Pharmaceutical tablets (Modalert and Diazepam), cannabis (herbal and resin) and herbal samples were not further analysed. The powders and crystal samples were weighed separately, labelled and analysed without any initial purification. Samples (30 mg) were dissolved in deuterated solvent (0.6 mL) for NMR spectroscopy, as well as being analysed by ESI-MS. Complete assignments of ^1H and ^{13}C NMR chemical shifts were achieved by means of 1D and 2D NMR techniques, including H-H COSY, HSQC, HMBC, and DEPT experiments. Further 2D NMR (^{15}N HMBC, ^{19}F NMR, H2BC) experiments were run in some cases for additional investigation. Detection of adulterants and drug determination against authentic standards were also performed using NMR and ESI-MS.

3.3. Results and discussion

3.3.1. Samples description and physical profiling

Amnesty bins can be a valuable source of information in relation to route of administration, packaging system, doses and drugs availability, e.g. powder, tablets, capsules, herbs, liquid, and stamp. Samples in the amnesty bins were received in small plastic bags, folded paper, bomb (twisted cigarette paper) and capsules. Tools such as cannabis grinders, snorters, and spoons were found, some examples are shown in Fig. 1. These tools might give information about the method of administration, e.g. two ketamine samples were placed with inhalers (Fig. 1, c). The most common administration route of illicit ketamine is the intranasal method by snorting the powder of ketamine [24]. Drugs that are twisted in a cigarette paper suggest oral administration. Also, each of these small twisted samples is one dose of the drug. Samples in a yellowish liquid form are the freebase of the drug, while a salt is supplied either as crystals or as powders. The freebase form is more likely to be smoked as it is much more volatile than the corresponding salt, i.e. crack cocaine

is usually smoked, and cocaine (salt) is more likely to be injected or snorted. This analysis of amnesty bins will focus in detail on uncommon and new drugs.



Fig. 1. Samples as received in the amnesty bin in (a) small plastic bags, (b) folded paper, tablets, (c) inhalers, and (d) tools such as cannabis grinders

3.3.2. Analysis of a 2013 Bristol amnesty bin

Most samples in the Bristol amnesty bin (2013) were crystals or powdered forms 88% (196 samples) compared with 24 tablets (11%) and only 3 liquid samples (1%) (Fig. 2). A total of 13 different drug types from Classes A and B as well as drugs under the Psychoactive Substances Act (2016) were found in the Bristol amnesty bin (Table 1). Despite the emergence of further new NPS, established illicit drugs such as cocaine and MDMA are still readily available and popular. Class A drugs represent 75% of the Bristol amnesty bin contents. A crystalline form of MDMA was clearly the dominant drug 64.3% (133 samples) (Fig. 3) alongside 2.9% (6) MDMA in tablet form. Although MDMA first appeared in the UK in 2009, this

drug is still very popular as a party drug. Indeed, MDMA users in the United Kingdom are reported to be the highest in Europe [25].

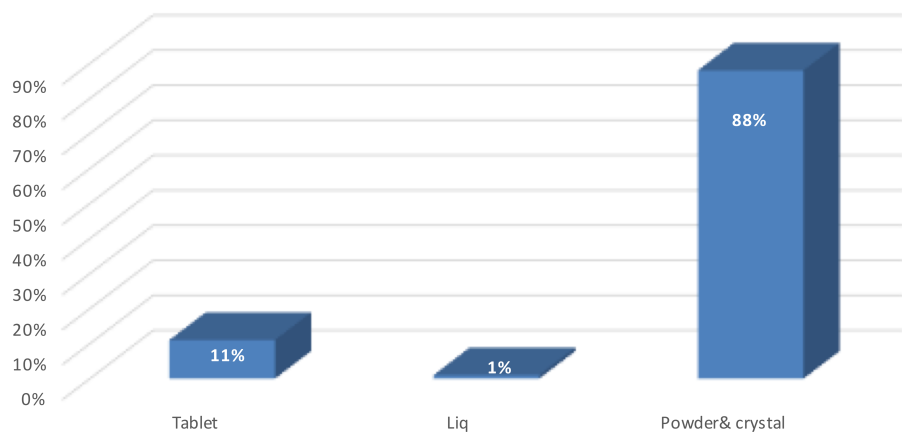


Fig. 2. Physical forms of substances identified in the Bristol amnesty bin

Table 1

The number and percentage occurrence of different drugs and their classes found in the Bristol amnesty bin

	Name	Number	Percentage
Class A	Cocaine (salt)	6	2.9%
	Crack cocaine	1	0.5%
	MDMA (crystals)	133	64.3%
	MDMA (tablet)	6	2.9%
	2C-B	1	0.5%
	Amphetamine	2	1%
	DMT	1	0.5%
	LSD	1	0.5%
	Flephedrone	4	1.9%
Class B	Mephedrone	13	6.3%
	Ketamine (salt)	19	9.2%
	Cannabis	-	-
Psychoactive Substances Act (2016)	6-DMAPB	1	0.5%

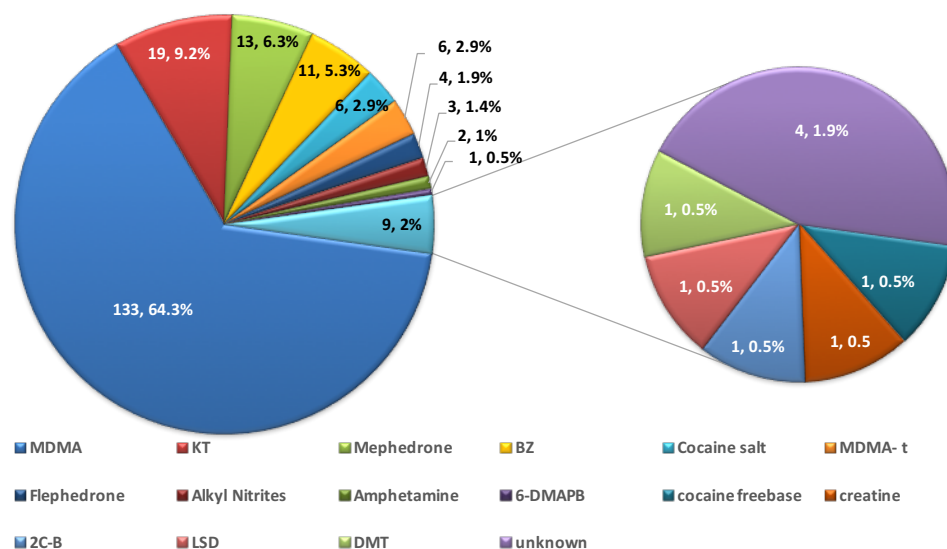


Fig. 3. Bristol amnesty bin: sample names, numbers, and their percentage occurrence

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is typically available in crystal form as the hydrochloride salt of the racemate. In tablet form, MDMA contains other compounds, including excipients, e.g. tableting agents, binders; in both tablet and crystal forms there are potentially (and potentially toxic) adulterants [26]. The synthesis of MDMA usually begins with a starting material containing a methylenedioxyphenyl ring, e.g. safrole, isosafrole, piperonal, or piperonylmethylketone (PMK) [27] and their possible impurities, e.g. of the aldehydes their corresponding carboxylic acids and even their primary alcohols, may be carried through. The so-called golden ecstasy may possibly be a result of using isosafrole or safrole as a starting material. MDMA tablets identified in the amnesty bin displayed a variety of different shapes, sizes, colours, and logos (Fig. 4). MDMA samples in crystalline form were found to be highly pure by NMR spectroscopy (Fig. 5). A total of 6 tablets were found to contain MDMA; identification followed ^1H NMR and was confirmed by ESI-MS. Chemical shifts, coupling constants, number of protons, and peak multiplicity of MDMA were measured: ^1H NMR (500 MHz, D_2O) δ 1.27 (d, $^3J_{\text{HH}} = 6.5$ Hz, 3H, H-3'), 2.71 (s, 3H, H-4' (N-CH₃)), 2.82 (dd, $^2J_{\text{HH}} = 14.0$, $^3J_{\text{HH}} = 6.5$ Hz, 1H, H-1' (a)), 2.97 (dd, $^2J_{\text{HH}} = 14.0$, $^3J_{\text{HH}} = 6.5$ Hz, 1H, H-1' (b)), 3.47 (sextet, $^3J_{\text{HH}} = 6.5$ Hz, 1H, H-2'), 5.95 (s, 2H, H-3), 6.78 (d, $^3J_{\text{HH}} = 8.0$, 1H, H-5), 6.82 (s, 1H, H-2), 6.87 (d, $^3J_{\text{HH}} = 8.0$ Hz, 1H, H-4). MS data in positive ion mode gave $[\text{M}+\text{H}]^+$ found 194.1184, $\text{C}_{11}\text{H}_{16}\text{NO}_2$ requires 194.1181. MDMA crystal samples were also analysed with an ALPHA-ATR-FTIR and the sample identity confirmed by matching the spectrum with the TICTAC database (Fig. 6).



Fig. 4. MDMA crystalline forms (upper) and tablets displaying different logos and shapes (lower)

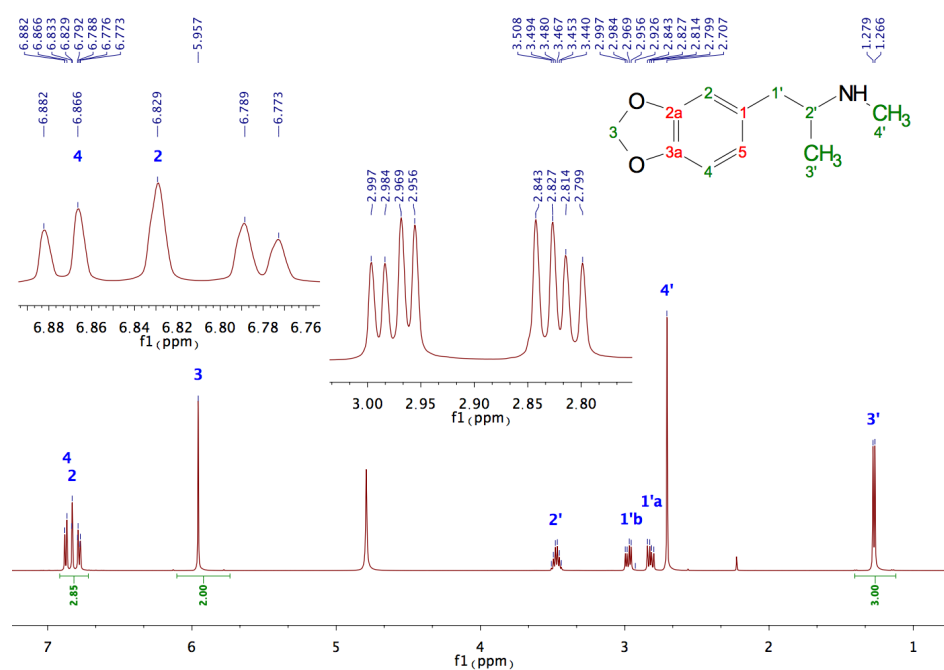
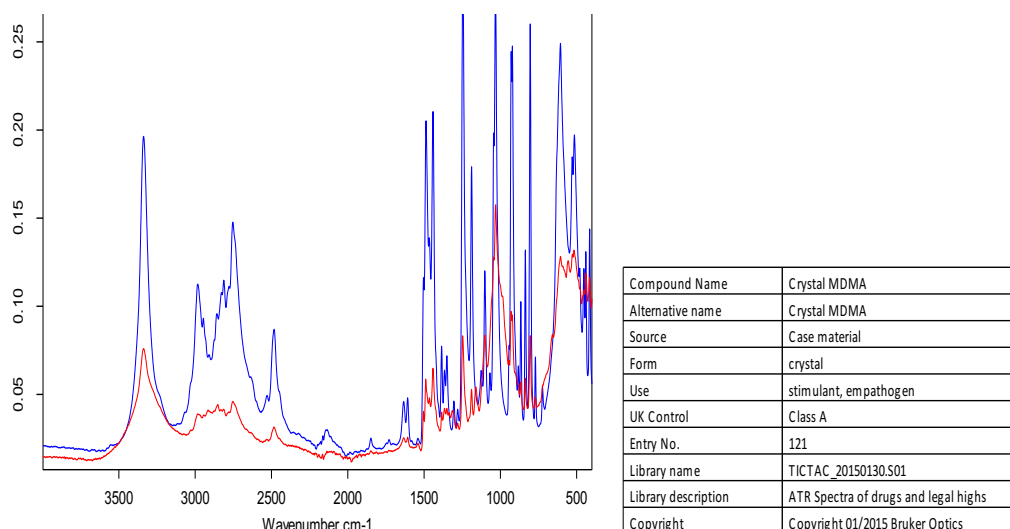


Fig. 5. ^1H NMR assignment of MDMA (D_2O)



Color	Content [%]	Compound name	CAS Number	Molecular formula	Molecular weight
	35.9	Crystal MDMA			

Fig. 6. ATR-FTIR spectrum of a crystal sample of MDMA (blue), and the matching MDMA spectra from TICTAC database (red), together with the MDMA details

Ketamine, a drug recently upgraded from Class C to Class B, was present at 9.2% (19 samples) as its salt. Cathinone derivatives, mephedrone (methcathinone) and its fluorinated derivative, flephedrone (4-fluoromethcathinone) were detected at levels of 6.3% (13 samples) and 1.9% (4 samples) respectively (Fig. 3). In the case of mephedrone, 9 out of 13 samples were cut with monosodium glutamate (MSG). All the flephedrone samples were cut with benzocaine. Two samples (1%) of amphetamine (*N*, α -methylphenethylamine), a synthetic phenethylamine, were identified in the amnesty bin. Both samples contained caffeine and (table) sugar as adulterants. Other samples found as fine white powders contained cocaine salt 2.9% (6 samples). One brownish sticky sample (0.5%) of crack cocaine (freebase) was detected; this freebase having been prepared by neutralisation of the salt.

White powders, simply presented in folded paper, were identified as dimethyltryptamine (DMT) freebase, a hallucinogenic drug. Such tryptamine is substituted indole compounds (Fig. 7a) and occur naturally in several plants. They display pharmacological effects similar to those of LSD and magic mushrooms [28]. DMT is a Class A controlled drug (CD), first synthesized in 1931 [29].

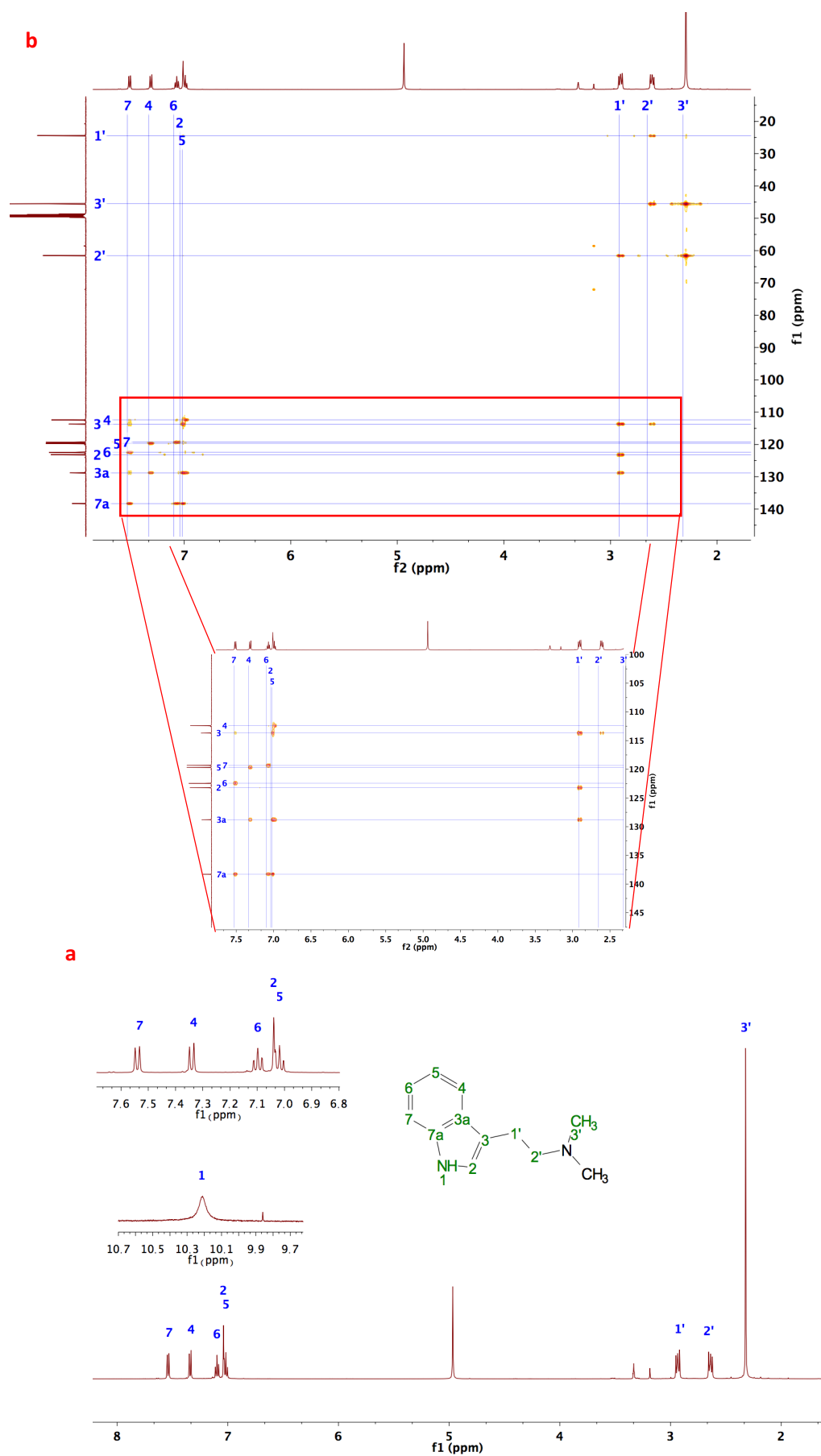


Fig. 7. (a) ^1H and (b) HMBC NMR of DMT (CDCl_3 , 50 mg/mL, 500 MHz) with its assignments

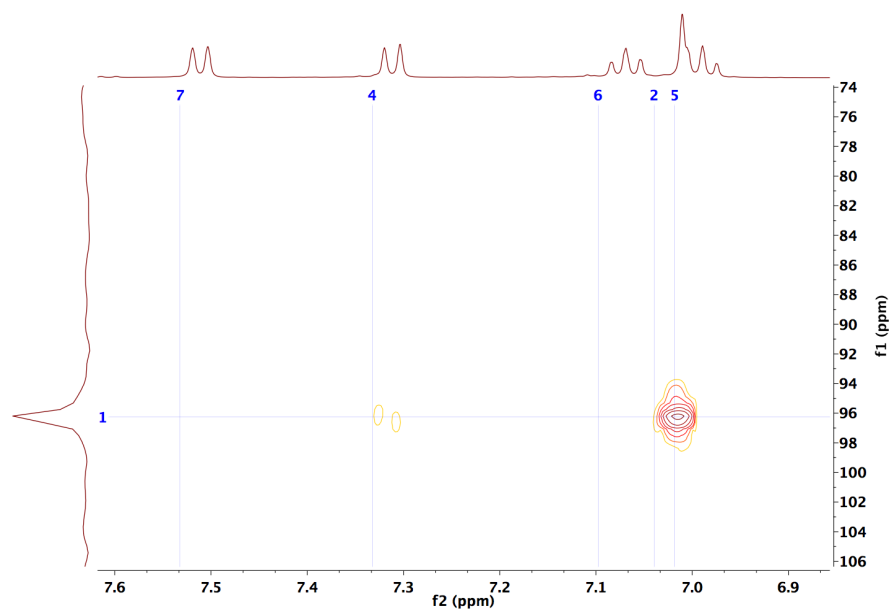


Fig. 8. ^{15}N HMBC showing the coupling between NH and H-2

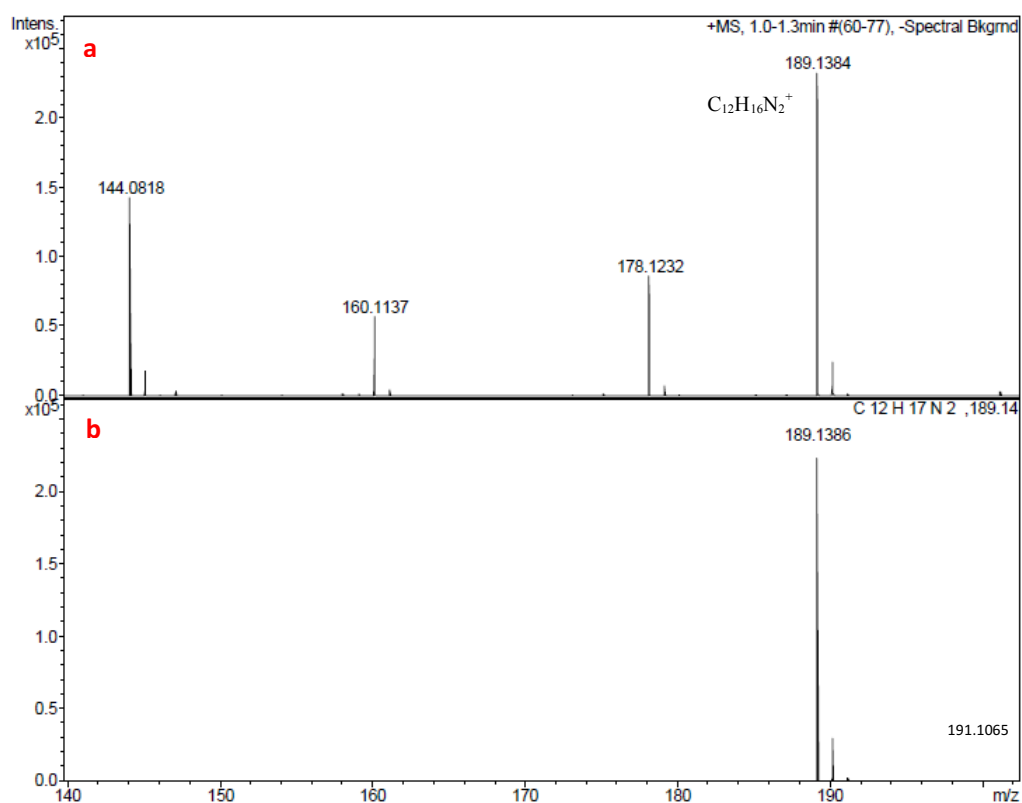


Fig. 9. (a) ESI-MS spectrum of extract ion mass of DMT and
(b) its simulated pattern

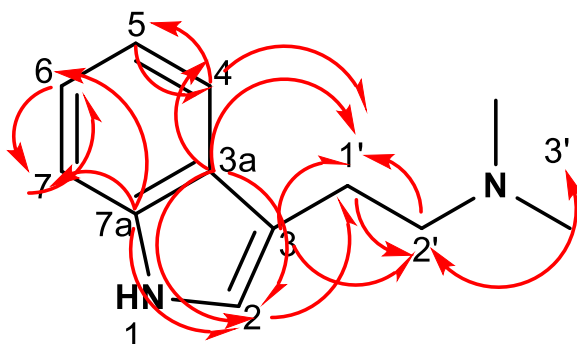


Table 2

Position	¹ H	¹³ C	HMBC (C→H)	
			² J	³ J
1'	2.88-2.93(m, 2H)	24.2	H2'	
2'	2.58-2.64 (m, 2H)	61.4	H1'	H3'
3'	2.29 (N-(CH ₃) ₂)	45.4		H2', H3'
1	10.20 (s, NH)			
2	7.07 (s, 1H)	123.0		H1'
3	-	113.5	H1', H2	H2'
3a	-	128.6	H4	H2, H1'
4	7.31 (d, ³ J _{HH} = 7.5 Hz, 1H)	112.2	H5	H1'
5	6.99 (t, ³ J _{HH} = 7.5 Hz, 1H)	119.1	H4	
6	7.92 (t, ³ J _{HH} = 7.5 Hz, 1H)	122.2	H7	
7	7.51 (d, ³ J _{HH} = 7.5 Hz, 1H)	119.5	H6	
7a	-	138.1	H7	H2, H6

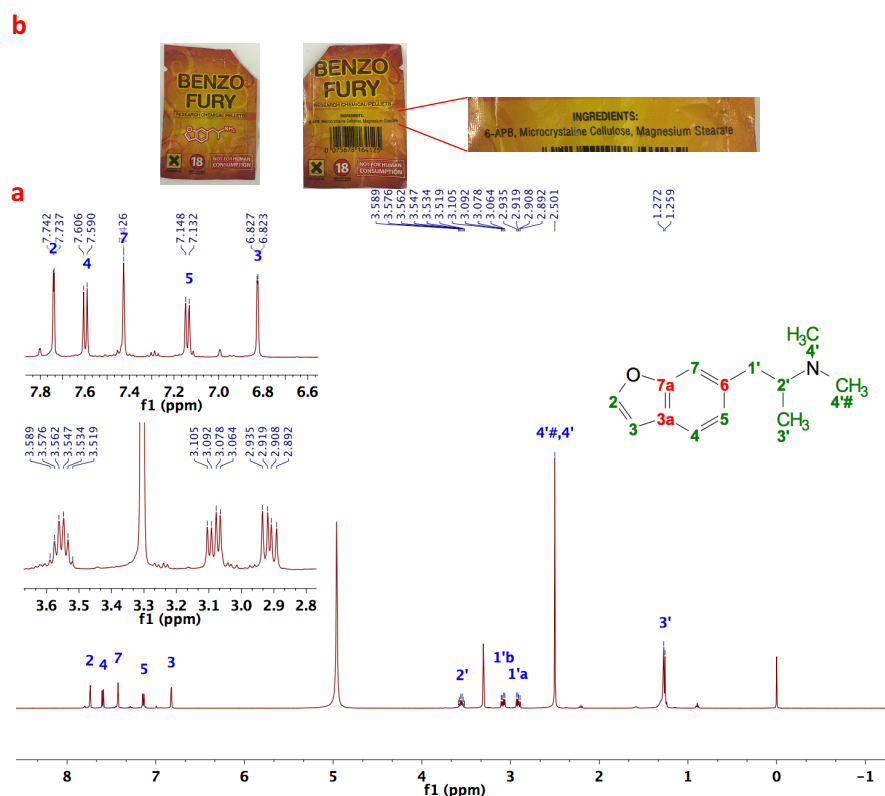


Fig. 11. (a) 6-DMAPB NMR assignments and (b) the package in which it was found

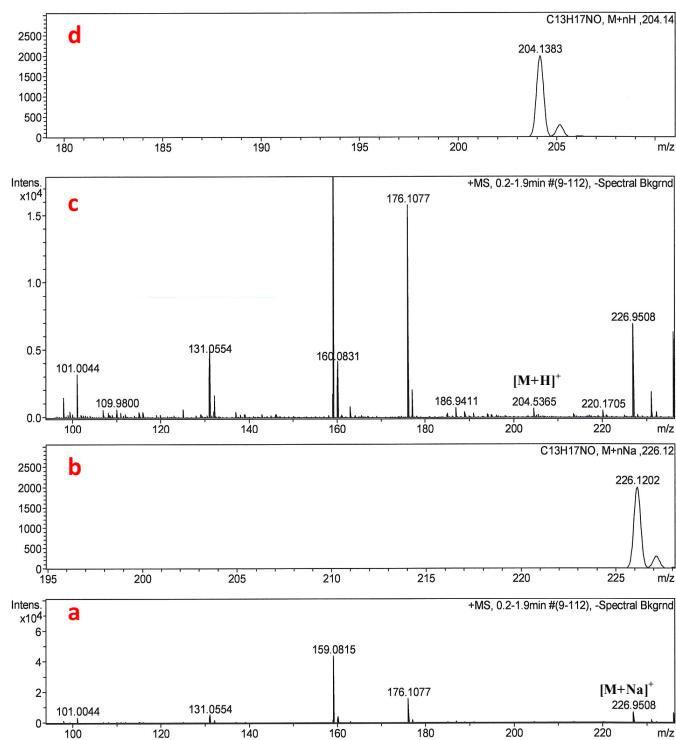


Fig. 12. (a) MS spectrum of 6-DMAPB with proposed possible fragmentations, (b) simulated pattern of $[M+Na]^+$, (c) expansion of the $[M+H]^+$ MS ion, and (d) simulated pattern of $[M+H]^+$

Indeed, tryptamines like DMT are substituted indole compounds which are both naturally occurring and synthetic. Many tryptamines, including DMT and LSD, have hallucinogen properties and are therefore listed as Class A. DMT is present in many plants and their seeds, including *Mimosa hostilis*, *M. teniflora* Willd. Poir, and *Psychotria viridis*. DMT can be abused by smoking, injecting, or ingestion either of the plant material or their crude or purified extracts, either alone or in combination with other extracts, e.g. Ayahuasca. Despite their controlled status, a number of DMT-containing natural products, including *M. hostilis*, are openly marketed on the internet. The historical use of DMT goes back many years to a tribe in the Amazon who boiled the plant, *M. teniflora* Willd. Poir, and then drank the resulting tea as a part of their tradition and religious customs. For these herbal and historical reasons, people might consider this drug as safe [28].

DMT was identified by total assignment of 1D and 2D NMR spectroscopic data (Fig. 7, Table 2), and comparing these with the literature [30]. The ^{15}N HMBC NMR was measured to determine the ^{15}N - ^1H coupling in the DMT (Fig. 8). The molecular formula was then confirmed by ESI-MS (Fig. 9). The key HMBC correlations in DMT is also illustrated in Fig. 10. ^1H NMR (500 MHz, methanol- d_4) δ 2.29 (s, 6H, H-3'), 2.58-2.64 (m, 2H, H-2'), 2.88-2.93 (m, 2H, H-1'), 6.99 (t, $^3J_{\text{HH}} = 7.5$, Hz, 1H, H-5), 7.07 (s, 1H, H-2), 7.92 (t, $^3J_{\text{HH}} = 7.5$ Hz, 1H, H-6), 7.31 (d, $^3J_{\text{HH}} = 7.5$ Hz, 1H, H-4), 7.51 (d, $^3J_{\text{HH}} = 7.5$ Hz, 1H, H-7), 10.20 (s, NH, H-1). ESI-MS (positive ion mode) $[\text{M}+\text{H}]^+$ found 189.1348, $\text{C}_{12}\text{H}_{16}\text{N}_2$ requires 189.1386 (1.1 ppm).

“Benzofury” is a brand name for 6-(2-aminopropyl) benzofuran (6-APB). This had been marketed as a “research chemical” or a “legal high” until it was banned, in May 2013, under a temporary class order [31], and rescheduled in May 2016 under the Psychoactive Substances Act [32]. Since 2011, 10 deaths and hospitalisations have been connected to the consumption of Benzofury in the UK [33]. The sample was commercially packaged as 6-APB with a note written on it “not suitable for human consumption”. However, detailed analysis of the NMR and MS data of the sample showed that the package contained 6-DMAPB and not the claimed (marketed) 6-APB (Fig. 11). This molecule has also not been previously reported in the literature. ^1H NMR (500 MHz, methanol- d_4) δ 1.26 (d, $^3J_{\text{HH}} = 6.5$ Hz, 3H, H-3'), 2.50 (s, 6H, 2 x CH_3 , H-4'), 2.91 (dd, $^2J_{\text{HH}} = 13.5$ Hz, $^3J_{\text{HH}} = 7.0$ Hz, 2H, H-1'(a)), 3.08 (dd, $^2J_{\text{HH}} = 13.5$ Hz, $^3J_{\text{HH}} = 7.0$ Hz, 2H, H-1'(b)), 3.64 (sextet, $^3J_{\text{HH}} = 6.5$ Hz, 1H, H-2'), 6.82 (d, $^3J_{\text{HH}} = 2.5$ Hz, 1H, H-3), 7.14 (d, $^3J_{\text{HH}} = 8.0$, Hz, 1H, H-

5), 7.42 (s, 1H, H-7), 7.69 (d, $^3J_{\text{HH}} = 8.0$ Hz, 1H, H-4), 7.73 (d, $^3J_{\text{HH}} = 2.5$ Hz, 1H, H-2). ESI-MS (positive ion mode) $[\text{M}+\text{H}]^+$ found 204.5365, $\text{C}_{13}\text{H}_{17}\text{NO}$ requires 204.1388 and for $[\text{M}+\text{Na}]^+$ found 226.9508 requires 226.1202 (Fig. 12). Furthermore, the sample is impure with signals from several impurities, these might be by-products, remaining starting materials, or/and solvents.

A folded piece of paper contained a crystalline drug that was identified as 2,5-dimethoxy-4-bromophenethylamine (2C-B), a ring-substituted phenethylamine that is similar to MDMA (Fig. 13, a). Since 2001, this drug was subjected to control under Class A in the UK. This designer drug, 2C-B is reported to be a powerful hallucinogenic substance besides its stimulant effects; the abuse of 2C-B has been reported [34]. Overdosing on this drug can cause frightening hallucinations besides hypertension and hyperthermia [34].

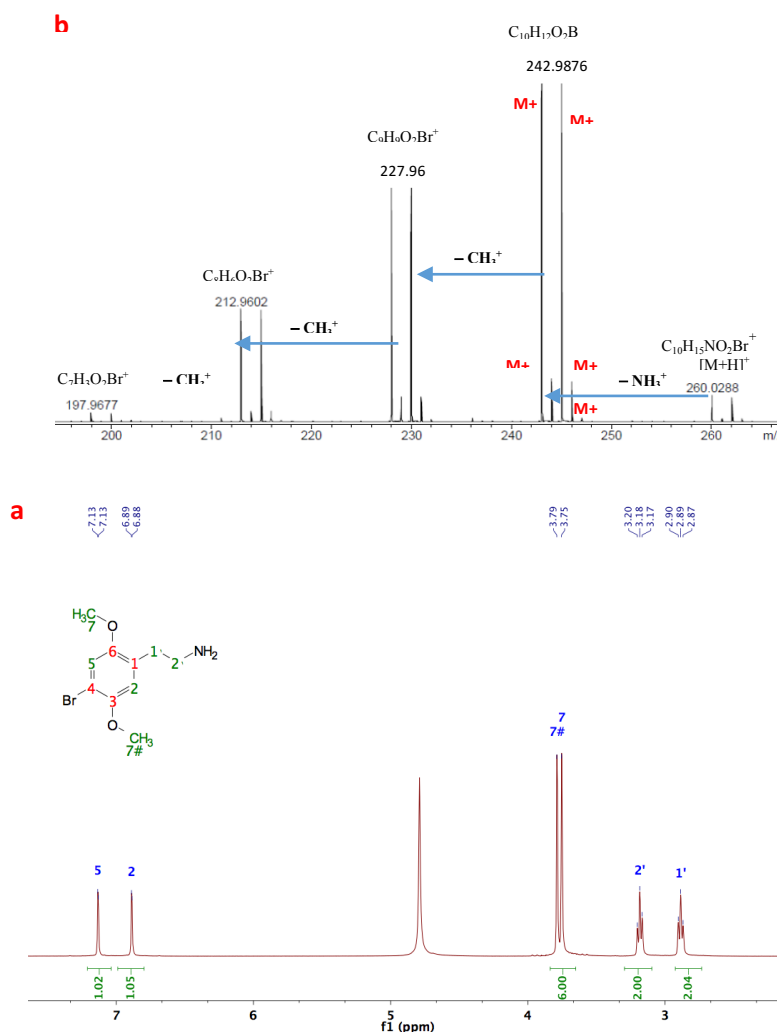


Fig. 13. (a) 2CB ^1H NMR and (b) ESI-MS spectrum

^1H NMR (400 MHz, D_2O) δ 2.89 (t, $^3J_{\text{HH}} = 4.0$ Hz, 2H, H-1'), 3.16 (t, $^3J_{\text{HH}} = 4.0$ Hz, 2H, H-2'), 3.75 (s, 3H, O-CH₃, H-7 (a)), 3.79 (s, 3H, O-CH₃, H-7 (b)), δ 6.78 (s, 1H, H-2), 7.13 (s, 1H, H-5) (Fig. 13, a); $[\text{M}+\text{H}]^+$ $\text{C}_{10}\text{H}_{15}\text{NO}_2\text{Br}$ found 260.0288, requires 260.0286 (0.7 ppm). The MS spectra of 2-CB (Fig. 13, b) is showing a unique 1:1 isotope pattern of ^{79}Br and ^{81}Br with the M+1, M+2, M+3, and M+4 isotopic peaks. A base peak (100%) at 242.9876 is due to the loss of ammonium group followed with the loss of methyl group from one of the methoxy substituents. Further loss of the second methyl group led to the ions at m/z 227.9648. This is followed with the ions at m/z 212.9602 as a result of the loss of another methyl group. The 1:1 $^{79:81}\text{Br}$ isotopic patterns are showing in all these ion fragments.

3.3.3. Analysis of the 2013 Glastonbury amnesty bin

Of 270 samples identified in the Glastonbury amnesty bin (Fig. 14), 86 (30.8%) were MDMA in tablet form, 77 (27.6%) were MDMA crystals, 41 (14.7%) were ketamine as powders and crystals, and 30 (10.8%) were cocaine powder. Three amphetamine samples (1.1%) were all cut with combinations of caffeine and sucrose. Two out of three samples of mephedrone were cut with MSG (1.1%).

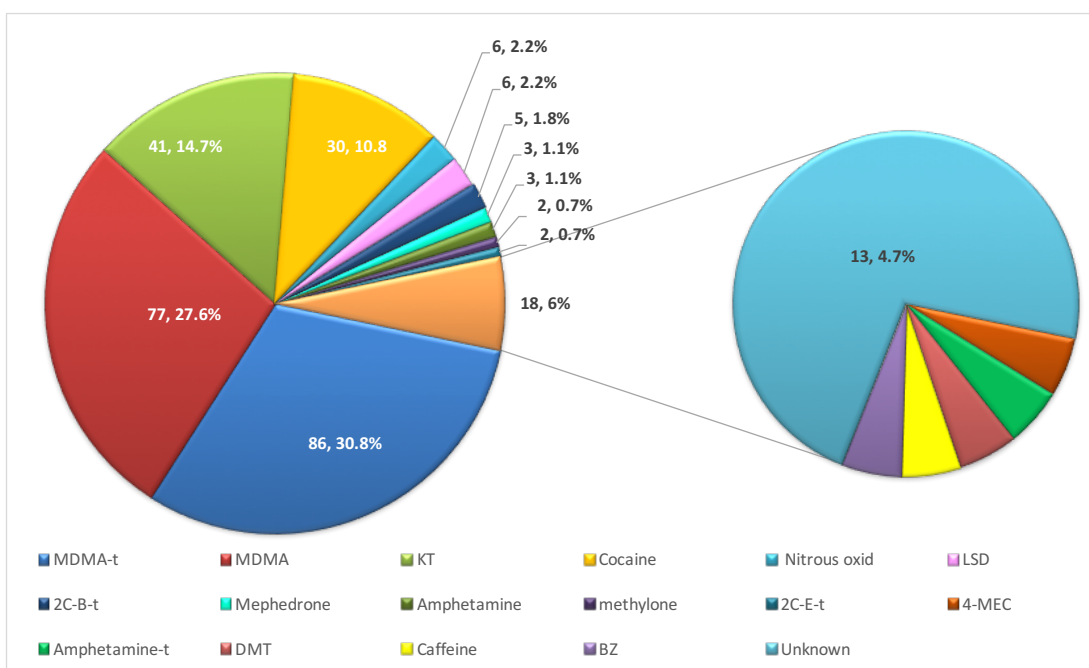


Fig. 14. A summary of the Glastonbury amnesty bin (2013) contents

A cathinone derivative, 4-methylethcathinone (4-MEC) was detected in one crystal sample (0.4%), cut with benzocaine. Another cathinone derivative was also found in the amnesty bin, methylone (2 samples, 0.7%). These cathinones have been marketed as relatives of mephedrone, known as “the second wave of synthetic cathinones” [35].

Two compounds from the 2C family of drugs were identified as 2CB (5 sample, 1.8%) and 2CE (2 samples, 0.7 %), both in tablet form. The composition of the 2CE street tablets was characterised by NMR and ESI-MS (Fig. 15, a). ^1H NMR (500 MHz, methanol- d_4) δ 1.15 (t, $^3J_{\text{HH}} = 10.0$ Hz, 3H, H-8), 2.60 (q, $^3J_{\text{HH}} = 10.0$ Hz, 2H, H-7), 2.91 (t, $^3J_{\text{HH}} = 5$ Hz, 2H, H-1'), 3.11 (t, $^3J_{\text{HH}} = 5.1$ Hz, 2H, H-2'), 3.78 (s, 3H, O-CH₃), 3.80 (s, 3H, O-CH₃), 6.78 (s, 1H, H-5), 6.80 (s, 1H, H-2); $[\text{M}+\text{H}]^+$ found 210.1492, $\text{C}_{12}\text{H}_{20}\text{NO}_2$ requires 210.1489 (1.6 ppm); $[\text{M}+\text{Na}]^+$ found 232.1263, $\text{C}_{12}\text{H}_{19}\text{NO}_2\text{Na}$ requires 232.1308 (19.3 ppm) (Fig. 15, b). Peaks at 0.90 (t), 1.28 (s), 1.58 (m), and 2.17 (t) are from a stearic acid ($\text{CH}_3-(\text{CH}_2)_{16}-\text{COOH}$), usually a magnesium or calcium stearate. It is a soap. Such supplements are often used in the manufacture of pharmaceutical tablets as lubricants and tableting agents to prevent ingredients from sticking to the equipment.

Two types of hallucinogenic drugs were identified in the Glastonbury amnesty bin, Lysergide (LSD) and DMT with 6 samples (2.2%) and 1 sample (0.37%), respectively. LSD is a very potent semi-synthetic hallucinogen and internationally controlled [36]. This kind of drug normally is supplied as or on a stamp (Fig. 16, c). The samples are prepared by dipping or spotting the dose in small square paper units (2-3 mm) characterised by different coloured designs. LSD is taken orally by placing the stamp or paper on the tongue. This CD is highly biologically active and therefore only very small (μg) doses are required. The dose is usually between 20-100 μg , but can be as high as 500 μg [37]. For analysis, the stamps were soaked in methanol for 24 h then submitted for the ESI-MS which confirmed their identity as LSD, in positive ion mode $[\text{M}+\text{H}]^+$ found 324.2055, $\text{C}_{20}\text{H}_{26}\text{N}_3\text{O}$ requires 324.2070 (4.6 ppm) (Fig. 16, a and b).

Caffeine and benzocaine were detected in two separate samples, both represented 1% of the samples in the amnesty bin. Benzocaine is a topical anaesthetic that can give a numbing effect similar to that caused by cocaine. This anaesthetic is a common adulterant of cocaine and might even be sold as cocaine.

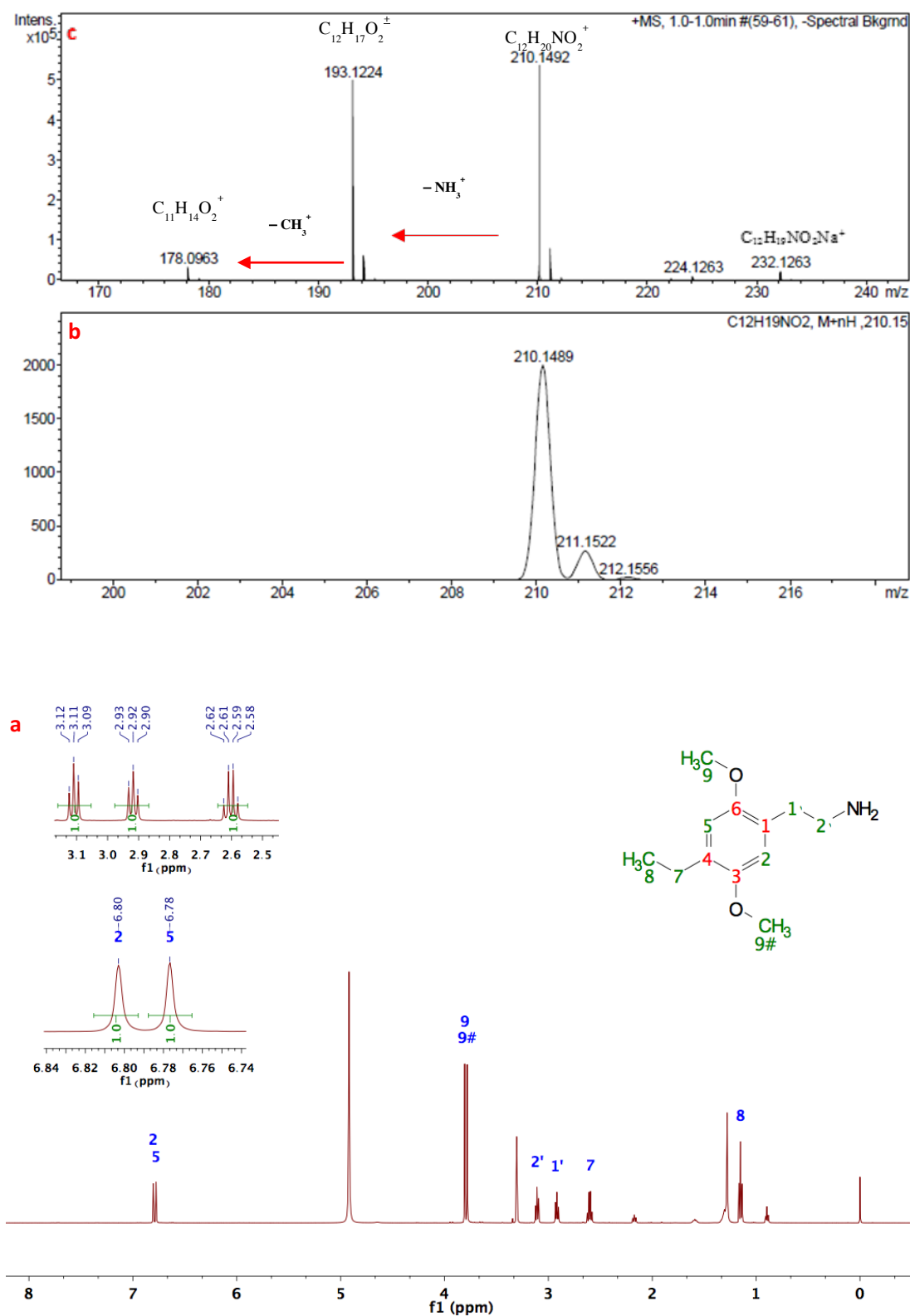
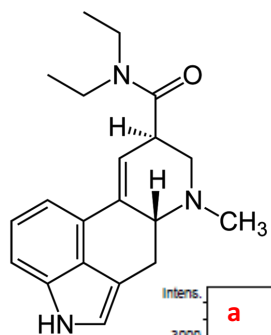


Fig. 15. (a) ^1H NMR assignment of the 2CE (30 mg/mL in methanol- d_4) with (b) its simulated MS pattern, and (c) the proposed MS fragmentations



c

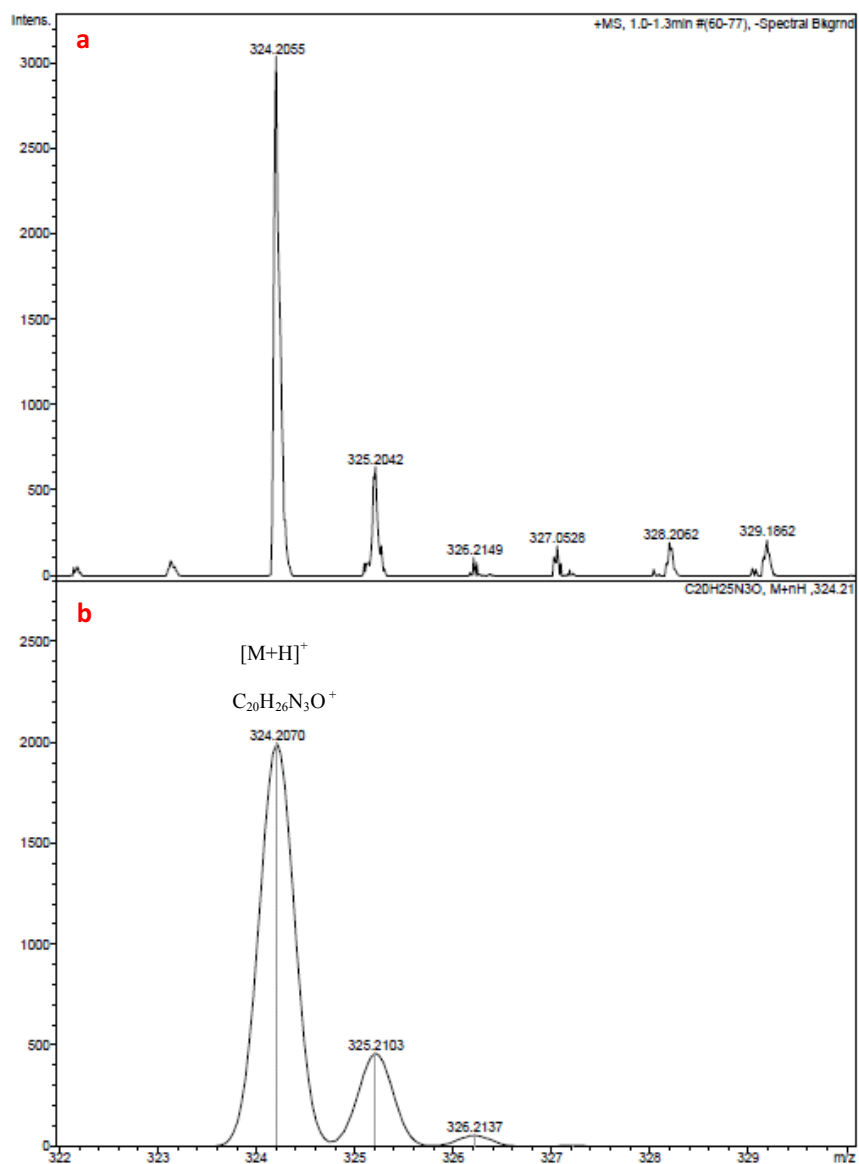


Fig. 16. (a) Extract ion MS of LSD with (b) its simulated MS pattern, and (c) LSD on stamps

A large quantity of diazepam (Class C) (123 samples) was identified in tablet form (Fig. 17, a). Illicit-drugs users attempt to take this kind of medication to help them “come down” after a heavy night (on “uppers”). Other medication found was Modalert (a 10 tablets strip), a psychostimulant drug. Nitrous oxide or “laughing gas” was found in the amnesty bin as well. People who abuse this gas are seeking a euphoric and relaxing feeling [38]. “Magic mushrooms” (0.9 g) were detected in the amnesty bin. Users abuse this mushroom because it has a hallucinogenic effect (Fig. 17). “Magic mushrooms” are a Class A CD which means that possession of this mushroom can result in up to seven years in jail [39].

Four clear to yellowish liquid samples (Fig. 17, d), in brown glass bottles, were found to contain alkyl nitrite, a highly volatile liquid. The samples were identified by ATR-FTIR and ^1H NMR (Fig. 18). Both isopropyl nitrate and isopropyl alcohol were detected in the liquid samples. In the streets, the alkyl nitrite is known as “poppers” and is available in “headshops”. The “poppers” are usually used in dance venues and can cause relaxation and a “rush”. The liquid sample can be isobutyl nitrite, isopropyl nitrite, *n*-butyl nitrite, nitrous acid butyl ester, or amyl nitrite, or a mixture of these [38].



Fig. 17. (a) Packets of diazepam tablets, (b) nitrous oxide metallic cylinders, (c) dried “Magic mushrooms”, (d) “poppers” found in the Glastonbury amnesty bin

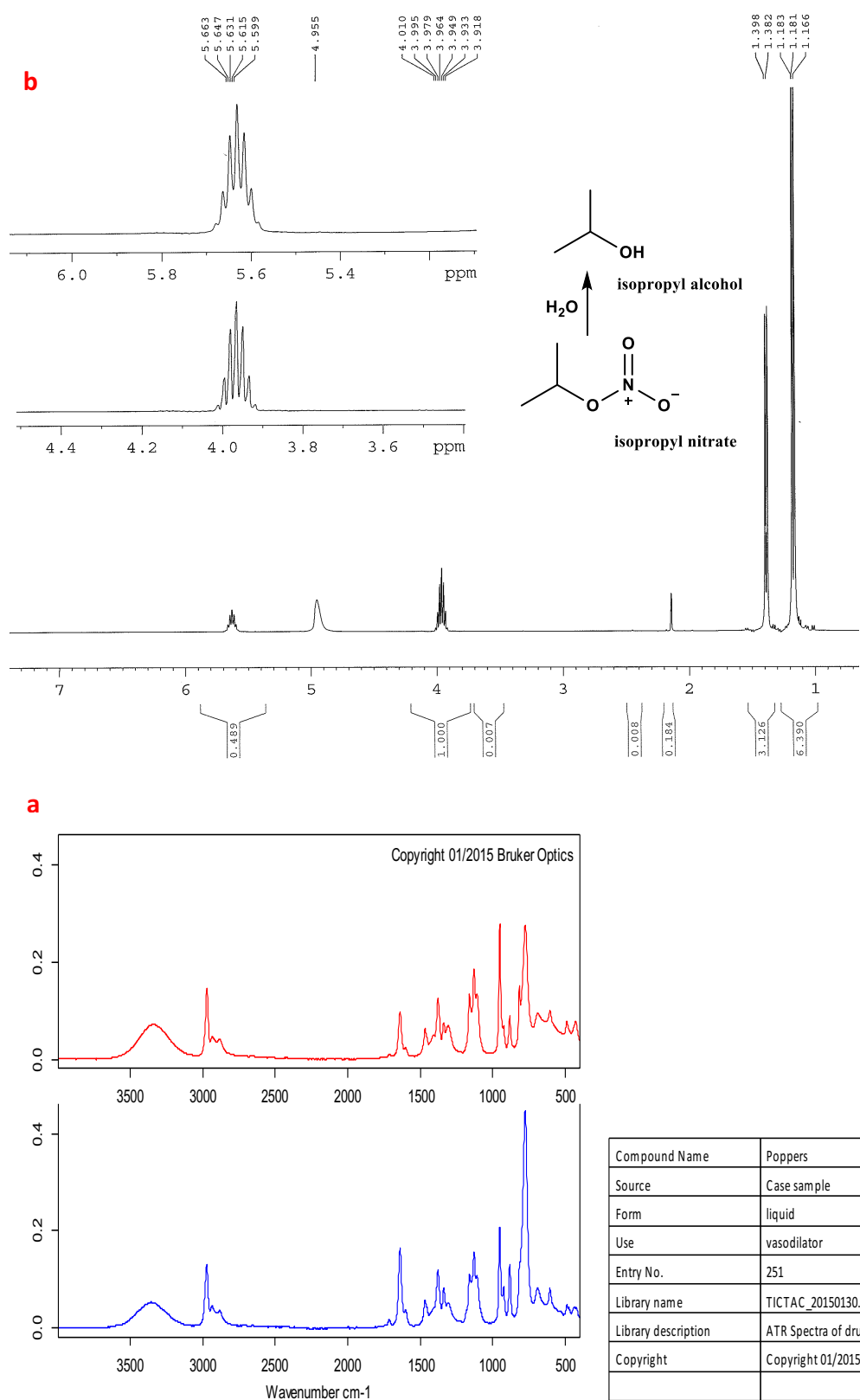


Fig. 18. Alkyl nitrite sample identified by (a) FT-IR using the TICTAC database and (b) ^1H NMR

3.3.4. Comparative analysis and profiling of a Bristol night club and Glastonbury amnesty bins (2013)

The analysis of amnesty bins covered samples from two different geographic locations (Fig. 19). The first one was from a night club in Bristol and the second from Glastonbury music festival, both in 2013. People who attend these kinds of festivals are mostly youngsters. The data showed similarities but with some interesting differences. The most noticeable similarity is that MDMA is still a drug of choice in both amnesty bins. However, the formulation was different, with the crystalline form more prevalent in the night club, but a more even mix of tablets and powders/crystals at Glastonbury. The data show that all ketamine samples were as salt forms, no freebase was detected. These were present in both amnesty bins with nearly 1.5-fold (14.7%) at Glastonbury compared to the Bristol night club (9.4%). This indicates the growing popularity of ketamine among young abusers. From these amnesty bin data, cocaine as a salt more prevalent among festival goers by nearly 4-fold (10.8% surrendered) compared to those samples from a Bristol night club (2.9%).

Locally, in the Bristol amnesty bin, mephedrone was the fourth most common drugs (6.3%). On the other hand, a variety of cathinone derivatives was found in the Glastonbury amnesty bin, including: mephedrone, methylone, and 4-MEC. Synthetic cathinones are reported to be the second largest illicit drugs group that are monitored by the EMCDDA with a total of 77 cathinone derivatives [40]. This group is characterised by the presence of a β -keto functional group on the side-chain of the phenethylamine (Fig. 20). In 2005, methylone, an MDMA derivative, was first reported as a synthetic cathinone in the Netherlands according to the EMCDDA [40]. The potent hallucinogenic drug LSD was present more in the Glastonbury amnesty bin (2.2%, 6 samples) compared to one sample in the Bristol night club amnesty bin. All the 2C-X family (2CE and 2CB) were identified as tablets. These are comparatively new to the black market and so are not yet widely available. Similar proportions of alkyl nitrites (“Poppers”) were present in both amnesty bins.

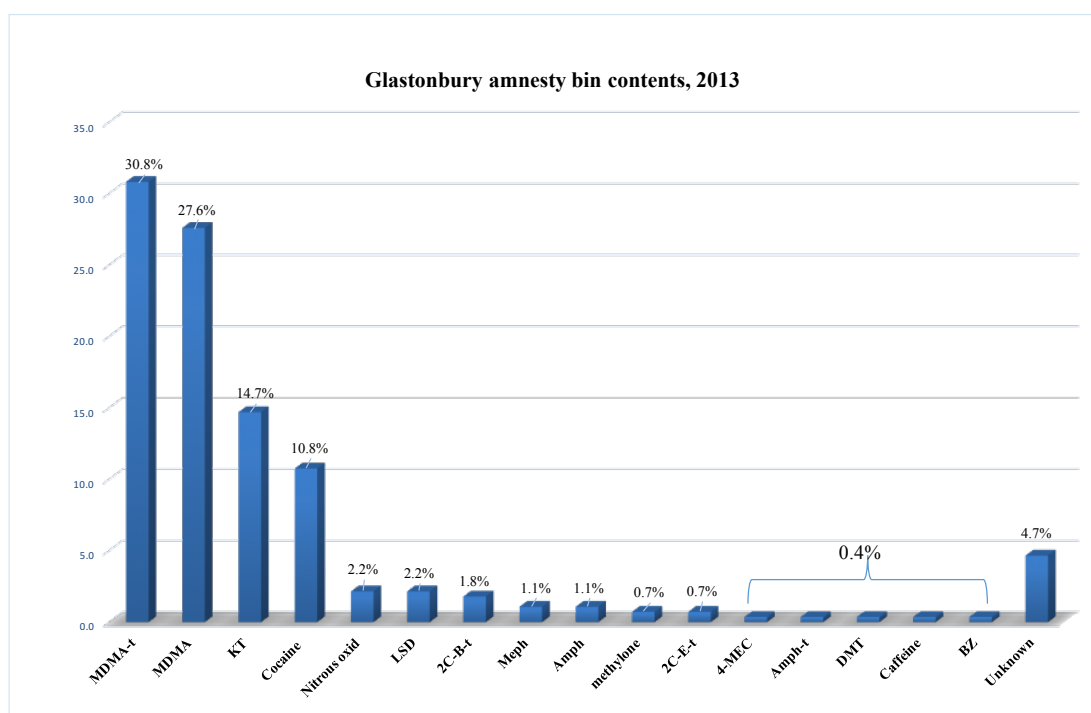
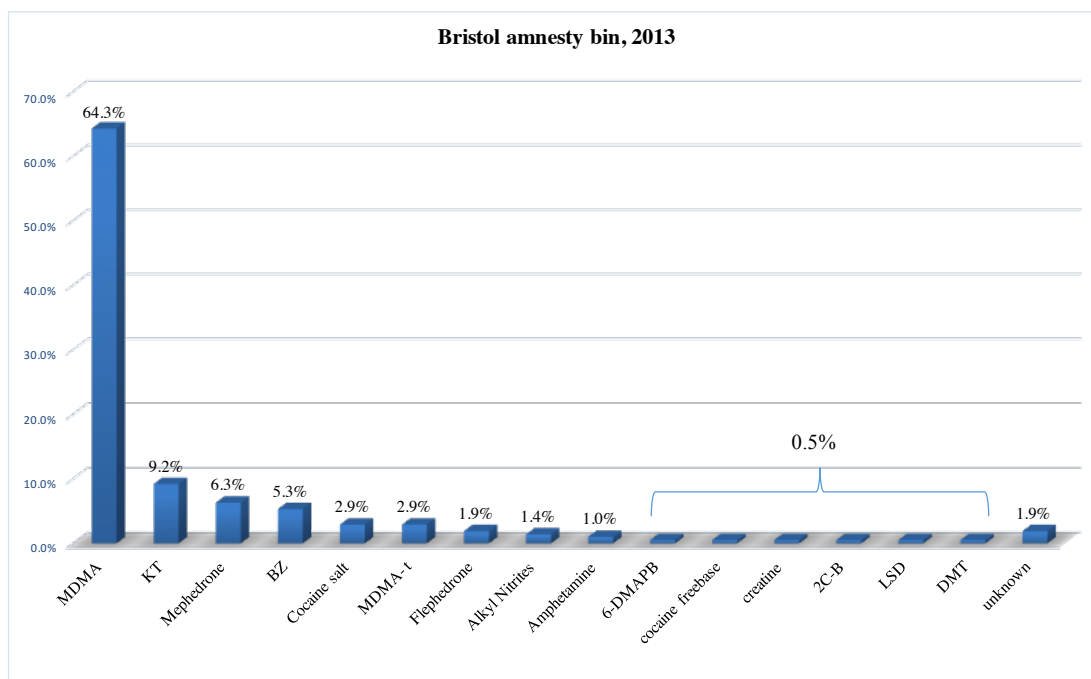


Fig. 19. Comparison between the contents of Bristol (upper) and Glastonbury (lower) amnesty bins (2013)

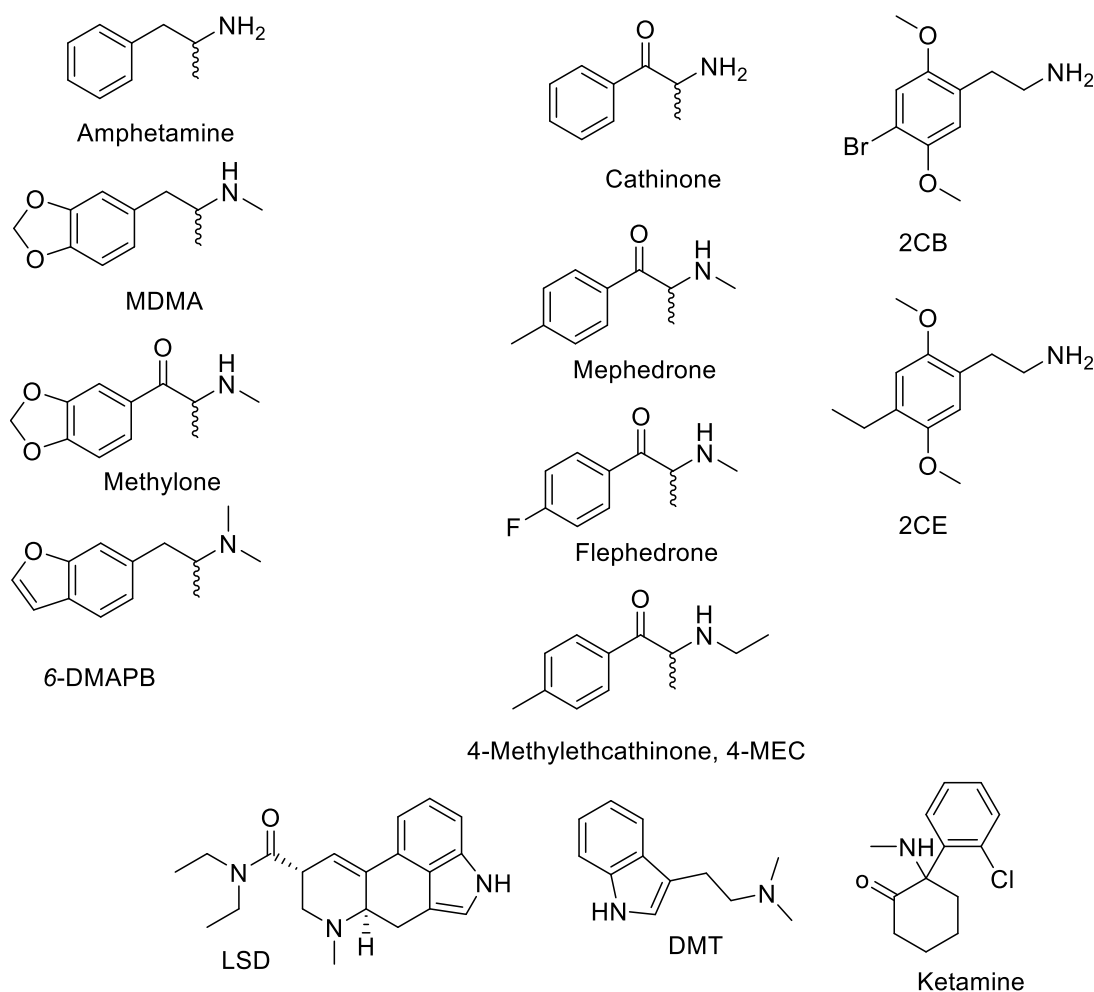


Fig. 20. Compounds identified in the Bristol and Glastonbury 2013 amnesty bins

3.3.5. Adulterants identified in the amnesty bins

To gain more profit and/or enhance the drug's effects, drug dealers usually add adulterants and diluents to the controlled substance [20]. The kind and the amount of the cutting against can be used to link between different samples and might link to different drugs seizers. For instance, flephedrone, a cathinone derivative, was found to be cut with benzocaine, a topical anaesthetic (Table 3). Benzocaine is a well-known cutting agent of cocaine and this might link the flephedrone to cocaine dealers [23]. Another suggestion is that benzocaine in the flephedrone samples can mimic the numbing effect of cocaine and might be sold as cocaine, and there is a concern about overdosing on redosing [24]. Mephedrone was also found to be impure and this drop in quality probably reflects the effect of recent control of cathinones [21]. Of the mephedrone samples, 9 out of 13 were cut with

monosodium glutamate (MSG) either as an easily accessible compound or due to it being similar in physical appearance. Ketamine samples were cut with stimulants, e.g. mephedrone and MDMA. Fillers such as sucrose and creatine were also used. These findings show the increasing popularity of ketamine. All amphetamine samples were cut with combinations of caffeine and sucrose. Few drugs were pure by NMR, but some were. This might be because a drug is not common, e.g. 2CB, 2CE, DMT. A few samples contained an adulterant or diluent alone, e.g. benzocaine, creatine, sugar, or caffeine.

Table 3

Adulterants identified in the amnesty bins

Sample ID	Adulterant
MDMA crystals	Sucrose
Amphetamine	Caffeine + sucrose
Cocaine	Benzocaine + sucrose
Mephedrone	MSG + creatine + sucrose + benzocaine + MDMA
4-MEC	Benzocaine
2CB	-
2CE	-
Ketamine	Methoxetamine + creatine + MDMA + mephedrone + mannitol + fructose
Methylone	-
DMT	-
Flephedrone	Benzocaine
6-DMAPB	Magnesium stearate, cellulose

3.3.6. Benchtop NMR (NMReady) evaluation for the analysis of illicit drugs in seized materials

Nanalysis manufactures a benchtop NMReady™ 60e spectrometer. The instrument is a compact, portable NMR with no liquid helium required, 60 MHz (1.4 T) operating frequency, 40:1 (ethyl benzene 1% single scan) sensitivity (SNR) and resolution at 50% = 1.2 Hz line-width. It has a permanent magnet and can be plugged directly into any normal wall power socket. One and two dimensional NMR can be measured on this machine, ^1H , ^{19}F , $^1\text{H}/^{19}\text{F}$ and COSY. The instrument offers simple data processing features. The NMReady™ 60e spectrometer was therefore evaluated for use in illicit drug analysis, three samples were tested on this instrument: flephedrone, ketamine, and cocaine (Figs. 21, 22, and 23 respectively) at 100 mg/mL in D_2O (1 mL) in standard 5 mm NMR tubes. All samples required such a high concentration.

The ^1H NMR of flephedrone (Fig. 21) can be identified. However, the spectroscopic data of other more structurally complex drugs, e.g. ketamine (Fig. 22) and cocaine (Fig. 23), were of poor quality that cannot be accurately or even easily interpreted. Furthermore, illicit samples in mixtures will be more complicated still and very difficult to analyse using this instrument. This instrument is more suitable for teaching, training, organic reaction monitoring, and for only really basic chemical analytical tasks.

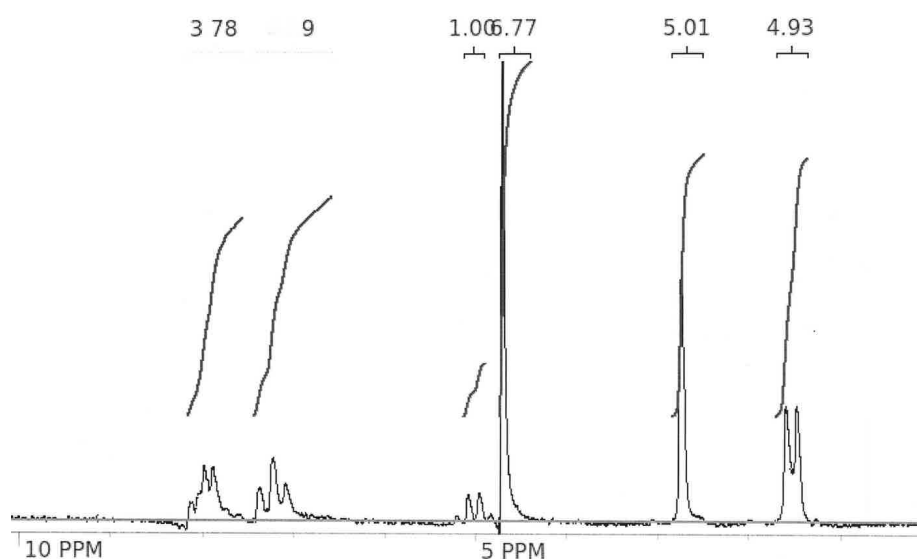


Fig. 21. Flephedrone

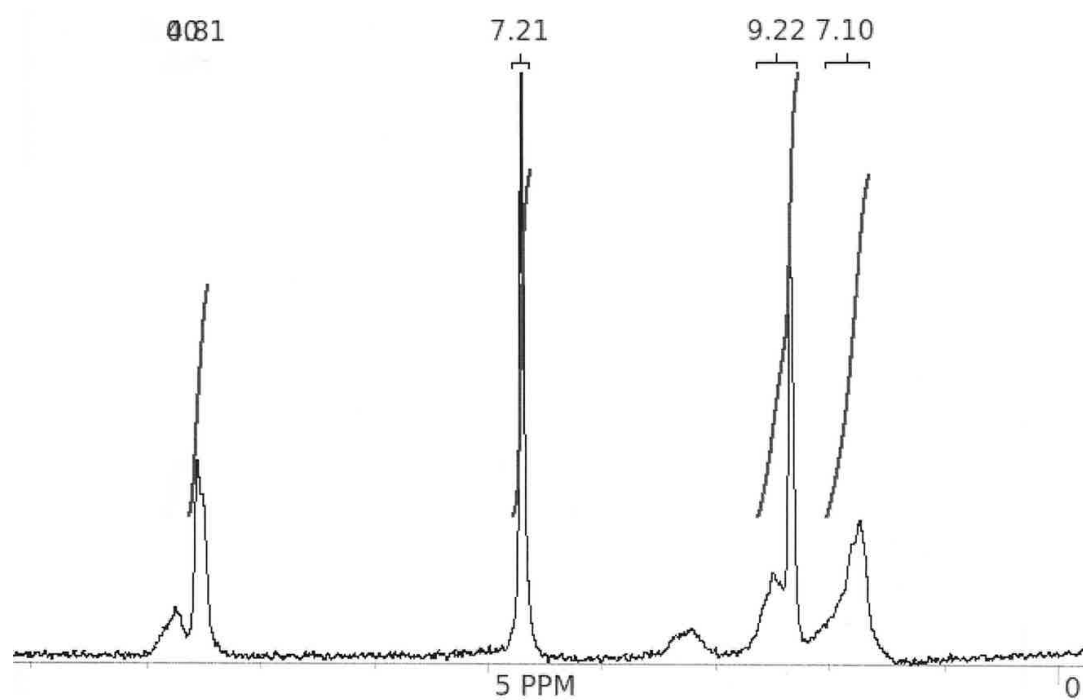


Fig. 22. Ketamine

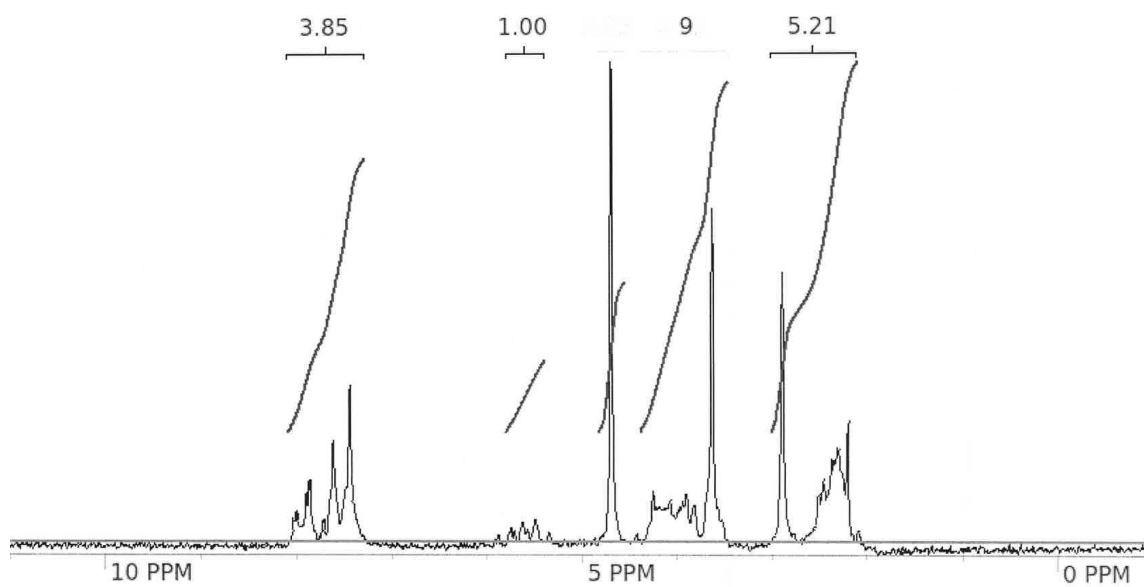


Fig. 23. Cocaine

3.4. Conclusions

Detailed analysis of amnesty bin contents can provide potentially useful information for the Police and law enforcement, specifically providing detail about currently abused drugs, drug availability, and their impurities. Class A CDs are still readily available, e.g. cocaine, MDMA. The latter was by far the most common drug in tablet or powder/crystalline form in both the night club and Glastonbury music festival amnesty bins, accounting for more than 58% of all samples tested. Mephedrone (readily available), flephedrone, and 4-MEC, cathinone derivatives, were found to be impure with benzocaine, sucrose, creatine, MDMA, and MSG. This confirmed the belief that drug purity is more likely to drop after biologically/pharmaceutically active compounds, e.g. legal highs, have been banned. Other drugs were found to be pure by NMR, the reasons for this are not clear, though each of the drugs is not common, e.g. 2CB, 2CE, DMT. The data show that ketamine was the second most common drug in both amnesty bins despite concern about harm associated with long term abuse of ketamine, with irreversible damage to the bladder.

These analytical results show the application of NMR spectroscopy in drug detection with no need for sample preparation, reference material, or internal standard. Police or forensic intelligence information may be gained from this kind of analysis to aid law enforcement. Findings from this analysis can provide information about the abuse of both illegal and legal drugs (medication), e.g. diazepam. Furthermore, identifying new drugs early can help predict potential health consequences of abuse. On the national level, a drug abuse map can be created by collaborating such drug disposal bin analysis with other findings from different regions in the UK. Internationally, a better picture can be provided about illicit drug trends by collaborating, sharing findings from amnesty bin analysis with other current information sources, e.g. waste water analysis, the Internet based system, Forensic Early Warning System (FEWS).

With minimum sample preparation, illicit drugs in crystal, powder, tablet, and liquid forms were successfully analysed by NMR spectroscopy. Using this technique for amnesty bin analysis allowed the vast majority of drugs, a few unknowns remain, including complex mixtures and cutting agents, to be correctly identified. This is in contrast with other analytical techniques, e.g. FT-IR, where mixture analysis is not accurate and requires a reference standard or database for comparison purposes.

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Chapter 4

Chemical assignments and impurity profiling of flephedrone

4.1. Introduction and overview

The close similarity between cathinone derivatives (Fig. 1) makes the specific identification of these drugs and their regioisomers challenging and could lead to mislabelling or accidental misidentification or deliberate deception as in the nature of the illicit cutting of street drugs. Flephedrone (4-fluoromethcathinone, 4-FMC) (Fig. 1c) was first synthesized in 1952, but surprisingly there is a paucity of published data for this compound. Compared with cathinone (Fig. 1a) and mephedrone (4-methylmethcathinone, 4-MMC) (Fig. 1b), the C-F bond on the aromatic ring of 4-FMC (Fig. 1c) has a specific polarity effect. This may influence receptor binding and therefore influence the biological activity [1, 2]. The biological effects of cathinone ring-substituted derivatives are claimed to be similar to cocaine, amphetamine, or MDMA (ecstasy) [3]. In the UK, a question about a decrease in the purity of cathinones has arisen after they were banned as Class B drugs under the Misuse of Drugs Act in April 2010 [4].

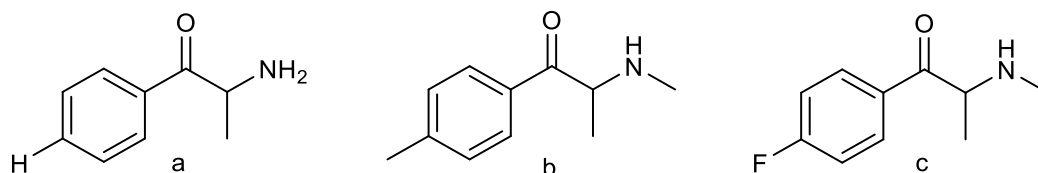


Fig. 1. (a) cathinone, (b) mephedrone, and (c) flephedrone

As routine analytical techniques, e.g. as used by the police, GC-MS [5, 6, 7] or Gas Chromatography-Flame Ionisation Detection (GC-FID) [6], infrared (FT-IR) [7], and Raman spectroscopy are widely used for the detection of controlled substances [8]. Nevertheless, standard reference materials for detecting what may be present in unknown samples are required. Using GC-MS alone would potentially not distinguish between the 2-, 3-, and 4-regioisomers of FMC, all the isomers having been reported in elegant synthetic work with full characterisation by Archer [7]. Electron ionisation-mass spectrometry (EI-MS) also leads to uncertainty in distinguishing between flephedrone

isomers [7]. More recently, screening methods for the detection of new (illicit) psychoactive drugs in urine were reported that use liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) [9], HPLC [6], and ultra-performance liquid chromatography-quadrupole time-of-flight-mass spectrometry (UPLC-QTOF-MS) [5].

NMR spectroscopy is an established analytical tool for the identification of controlled substances without the need for sample derivatisation or the need to remove any impurity. Also, distinguishing between regioisomers, between salt and free base, determining percentage purity and impurity ratios are successfully and rapidly achieved using NMR spectroscopy. Police or forensic intelligence information may be gained from this kind of analysis to aid law enforcement. The aim of this part of the research project is to develop a routine, rapid, and quantitative analytical approach to detect flephedrone as well as the impurities (cutting agents) present in street samples.

Four 4-FMC samples (from a Bristol nightclub, 2013) were analysed using ^1H , ^{13}C , ^{15}N HMBC, HSQC and ^{19}F observe NMR spectroscopy together with results from GC-FID, and Electrospray Ionisation-Mass Spectrometry (ESI-MS). Further investigation of the flephedrone and mephedrone aromatic regions using Nuclear Overhauser Effect Spectroscopy (NOESY) and Hetero-nuclear Overhauser Enhancement Spectroscopy (HOESY) NMR were performed. Analysis showed that they all contained benzocaine as the cutting agent, present in differing amounts from 5-12%. Using these methods, we successfully differentiated between flephedrone regioisomers and mephedrone in an analytical method validated for flephedrone as a substituted cathinone. These data show that this now illegal cathinone-derived stimulant (high) is now being cut and users cannot be certain of the purity of the drug they are taking. Furthermore, there are risks from the pharmaceutically active cutting agents themselves.

4.2. Experimental

4.2.1. Chemicals and materials

Four creamy coloured crystal samples, as part of an amnesty bin provided in a Bristol, UK nightclub (2013), were identified as flephedrone. A reference standard of 4-FMC HCl was purchased from LGC Standards, UK. An analytical standard of 4-MMC HCl was purchased from Aldrich, UK. Deuterium oxide (99.8 atom % D, Aldrich, UK), per-deuteriated methanol (99.8 atom % D, Cambridge Isotope Laboratories, USA), and all other solvents were of HPLC grade, $\geq 99.9\%$ purity (Aldrich, UK). Benzocaine B.P. was purchased from J. M. Loveridge plc (Southampton, UK).

4.2.2. Experimental procedures and instrumentation

Each sample of 4-FMC was weighed, dissolved, and analysed at 20-25°C without any purification. NMR spectra were recorded on Bruker 400 or 500 MHz spectrometers, referenced to residual protons in the deuteriated solvents. Samples (30 mg each) were vortexed for 2 min in D₂O (0.6 mL). Identification of 4-FMC samples and their impurities followed NMR analysis including: ¹H, ¹³C, ¹⁵N (HMBC), COSY, NOESY, HSQC, HMBC, HOESY, and ¹⁹F.

A GC-FID method was optimised to analyse authentic flephedrone and benzocaine B.P. dissolved in methanol (0.50 mg/mL). GC separation was performed on a CP-9003 (Chrompack, Middelburg, The Netherlands) using a capillary column ZB-WAX (polyethylene glycol, 10 m x 0.25 mm x 0.25 μ m, Phenomenex, UK). An injection volume (1-2 μ L) was carried by helium gas at a flow rate of 23-27 mL/s. The oven temperature was programmed with 250 °C injector and detector temperature, 110 °C (2 min) oven initial and 230 °C (4 min) final temperatures with 40 °C/min rate of heating, therefore the total run time was 9 min. The samples were injected directly into the GC-FID without any prior chemical derivatisation. Positive ion [M+H]⁺ mode MS was performed, on samples dissolved in methanol, using a Bruker Daltonics micrOTOF LC/MS equipped with an ESI source. Attenuated Total Reflection (ATR)-FTIR was equipped with the TICTAC database.

4.3. Results and discussion

4.3.1. ^1H NMR spectroscopy

Whilst the ^1H NMR spectrum of 4-FMC is similar to that of 4-MMC (Fig. 2), ring numbering is shown in Fig. 3, the absence of a 4-methyl aromatic substituent peak, and the coupling from the ^{19}F atom are diagnostic. The aromatic peaks in the ^1H NMR of mephedrone appear as doublets while in flephedrone they are dd and triplet due to coupling with the fluorine atom; ^1H , ^{13}C , and ^{19}F NMR spectral data are reported in Table 1. NMR spectra are further discussed and compared to the closely related cathinone derivative 4-MMC (Fig. 2). The structures were confirmed by comparing the NMR data with an authentic sample of flephedrone and the literature values [7], establishing the regioisomer as *para*-disubstituted. The ^1H NMR of flephedrone: (500 MHz, D_2O): δ 1.62 (d, $^3J_{\text{HH}} = 7.5$ Hz, 3H, H-3'), 2.81 (s, 3H, N-CH₃), 5.09 (q, $^3J_{\text{HH}} = 7.5$ Hz, 1H, H-2'), 7.35 (t, $^3J_{\text{HH}}$ $^3J_{\text{HF}} = 9.0$ Hz, 2H, 3 and 5), 8.10 (dd, $^3J_{\text{HH}} = 9.0$ Hz, $^4J_{\text{HF}} = 5.5$ Hz, 2H, 2 and 6). Mephedrone ^1H NMR (500 MHz, D_2O): δ 1.61 (d, $^3J_{\text{HH}} = 7.0$ Hz, 3H, 3'), 2.45 (s, 3H, 4-CH₃), 2.81 (s, 3H, N-CH₃), 5.08 (q, $^3J_{\text{HH}} = 7.0$ Hz, 1H, H-2'), 6.22 (d, $^3J_{\text{HH}} = 8.5$ Hz, 2H, 3 and 5), 7.92 (d, $^3J_{\text{HH}} = 8.5$ Hz, 2H, 2 and 6).

4.3.2. ^{13}C and ^{19}F NMR spectroscopy

The chemical structure of 4-FMC was supported by ^{13}C NMR spectroscopy with nine separate carbon signals evident (Fig. 4). The ^{13}C NMR of flephedrone is also compared with mephedrone. Flephedrone ^{13}C (500 MHz, D_2O): δ 15.2 (3'), 30.9 (N-CH₃), 59.5 (H-2'), 128.8 (C1), 132.4 (C2, 6), 116.4 (C3, 5), 167.7 (C-CH₃), 196.0 (CO), and mephedrone ^{13}C (500 MHz, D_2O): δ 15.3 (3'), 20.9 (4-CH₃), 30.8 (N-CH₃), 59.4 (H-2'), 129.0 (C2, 6), 129.6 (C4), 129.8 (C3, 5), 147.3 (C-CH₃), 197.1 (CO) ppm. Unfortunately, two lines in the literature ^{13}C NMR spectroscopic assignment table have been transposed [7], and the correct aromatic region assignment is given below in Table 1 for δ 116.4 (d, $^2J_{\text{CF}} 25.1$ Hz) and 132.4 (d, $^3J_{\text{CF}} 12.5$ Hz) ppm, where the coupling constants are diagnostic for *ortho* to F (C3, C5) and *meta* to F (C2, C6) respectively (Fig. 3). The ^{13}C - ^{19}F splitting patterns around the entire aromatic ring, e.g. 167.7 ppm, d, $^1J_{\text{CF}}$ 251.5 Hz; 128.8 ppm, d, $^4J_{\text{CF}}$ 2.5 Hz in the ^{13}C spectra (Table 1) are also significant. ^{19}F

NMR spectroscopy proves the presence and position of a fluorine atom in such samples. Comparing with the literature data [7], the ^{19}F NMR chemical shift values for the each of the four 4-FMC samples show *para*-substitution: ^{19}F NMR showed -102.1 ppm (tt, $^3J_{\text{HF}}$ 9.0 Hz, $^4J_{\text{HF}}$ 5.5 Hz) (Fig. 5) with the d $^1J_{\text{CF}}$ 251.5 Hz easily visible only in the ^{13}C NMR spectrum, not in the ^{19}F NMR spectrum due to the low (1.1%) ^{13}C natural abundance (Fig. 5).

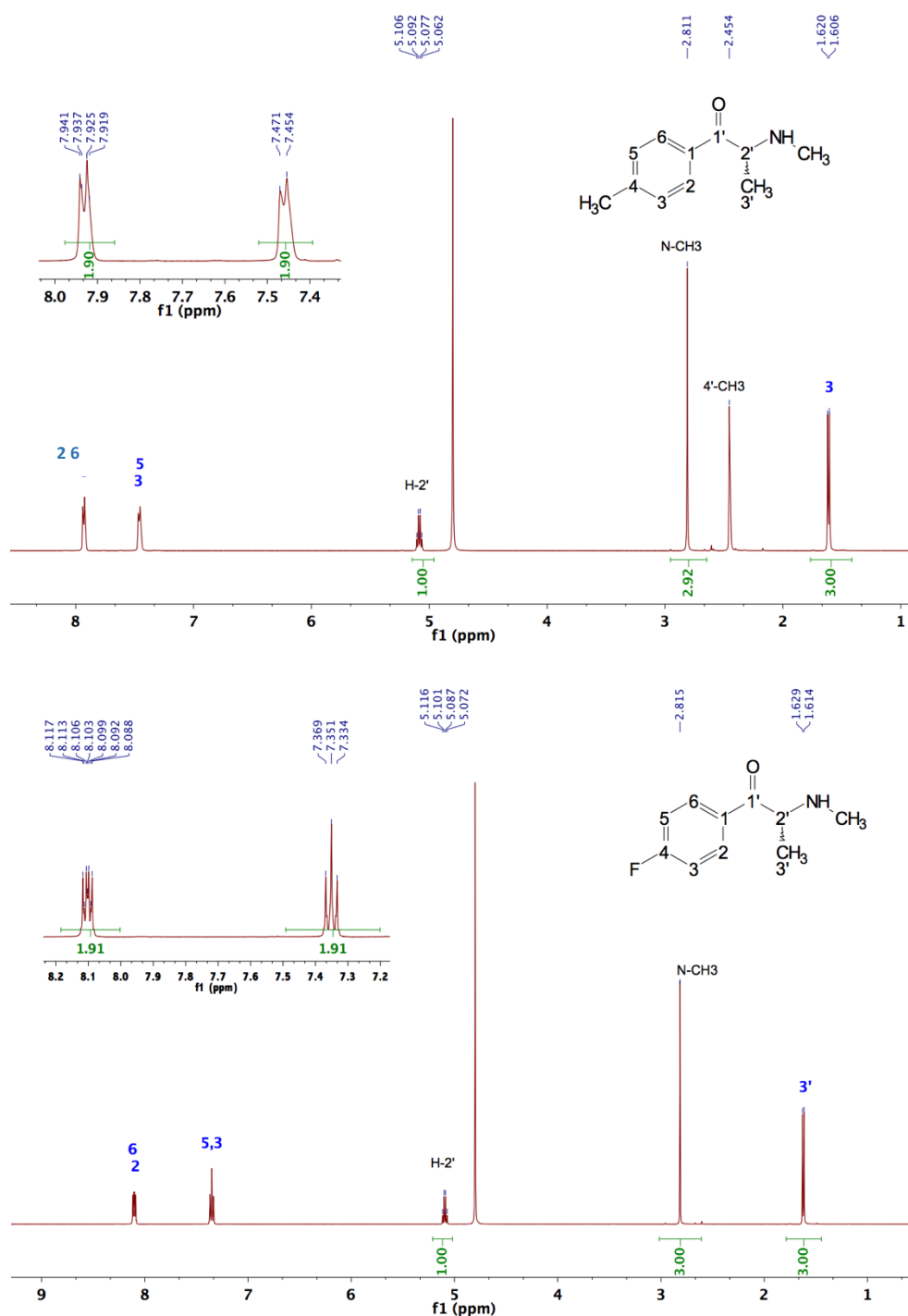


Fig. 2. ^1H NMR of mephedrone (upper) and flephedrone (lower) (D_2O)

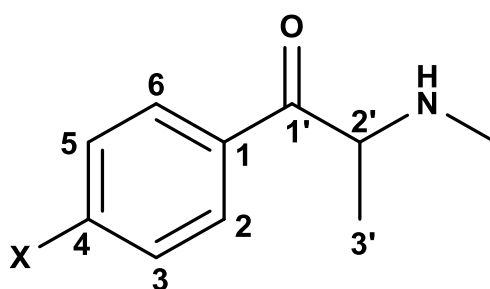


Fig. 3. Numbering of mephedrone (X = CH₃) and flephedrone (X = F)

Table 1

¹H (400 MHz), ¹³C (100 MHz), and ¹⁹F (470 MHz) spectral data of flephedrone (D₂O)

	Flephedrone			Mephedrone	
Position	¹ H	¹³ C	¹⁹ F	¹ H	¹³ C
1	-	128.8 (d, ⁴ J _{CF} 2.5 Hz)		-	147.3
2, 6	8.10 (dd, ³ J _{HH} 9.0 Hz, ⁴ J _{HF} 5.5 Hz)	132.4 (d, ³ J _{CF} 12.5 Hz)		7.94 (d, ³ J _{HH} 8.5 Hz)	129.0
3, 5	7.35 (t, ³ J _{HH} = ³ J _{HF} 9.0 Hz)	116.4 (d, ² J _{CF} 25.1 Hz)		7.47 (d, ³ J _{HH} 8.5 Hz)	129.8
4	-	167.7 (d, ¹ J _{CF} 251.5 Hz)	-102.1, tt, ³ J 9.0, ⁴ J 5.5 Hz	-	129.6
1'	-	196.0		-	197
H-2'	5.09 (q, ³ J _{HH} 7.5 Hz)	59.5		5.08 (q, ³ J _{HH} 7.0 Hz)	59.4
3'	1.60 (d, ³ J _{HH} 7.5 Hz)	15.2		1.61 (d, ³ J _{HH} 7.0 Hz)	15.3
N-CH₃	2.81, s	30.9		2.81, s	30.8
4-CH₃	-	-		2.45, s	20.9

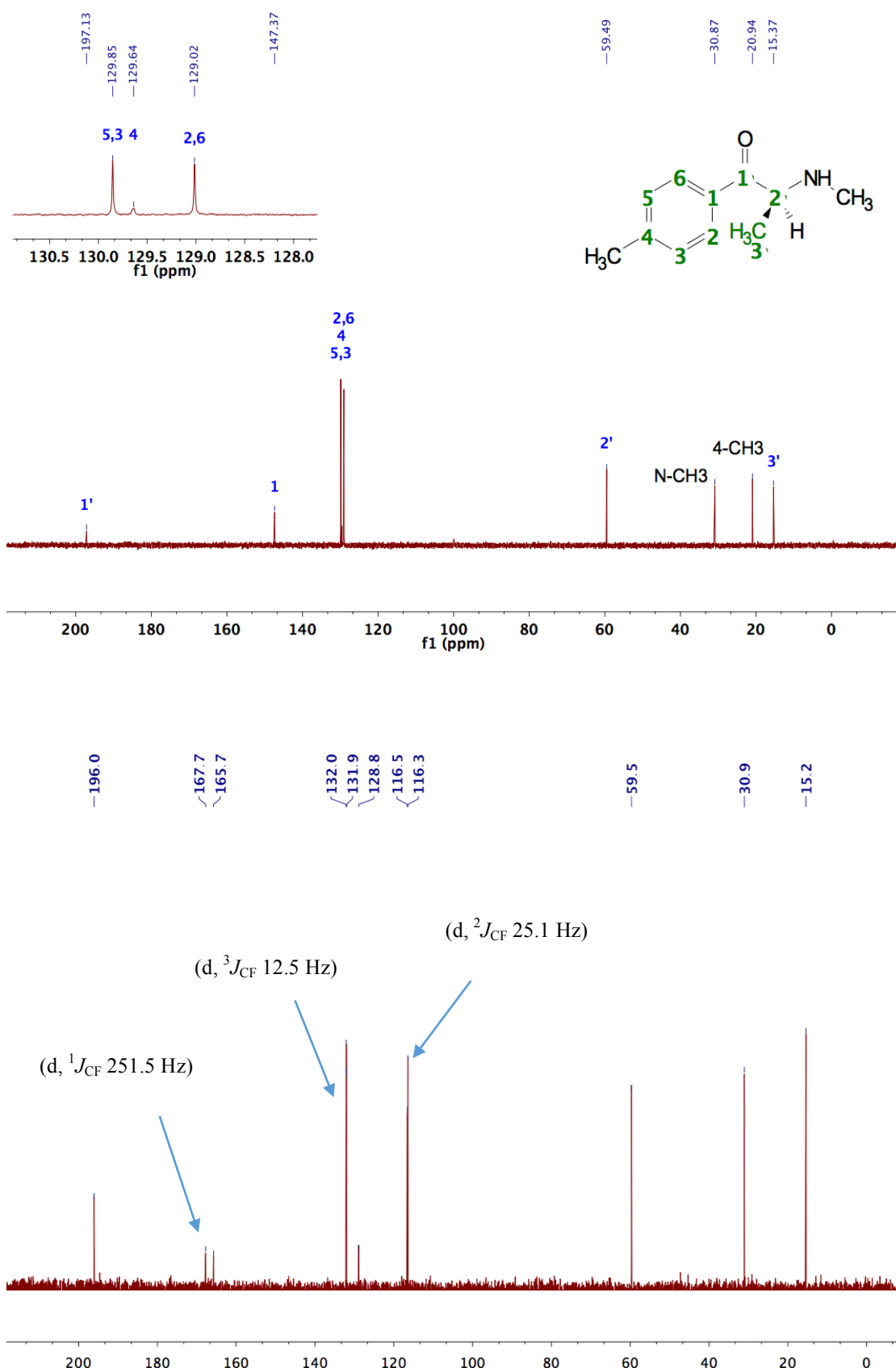


Fig. 4. ^{13}C NMR of mephedrone (upper) and flephedrone (lower)

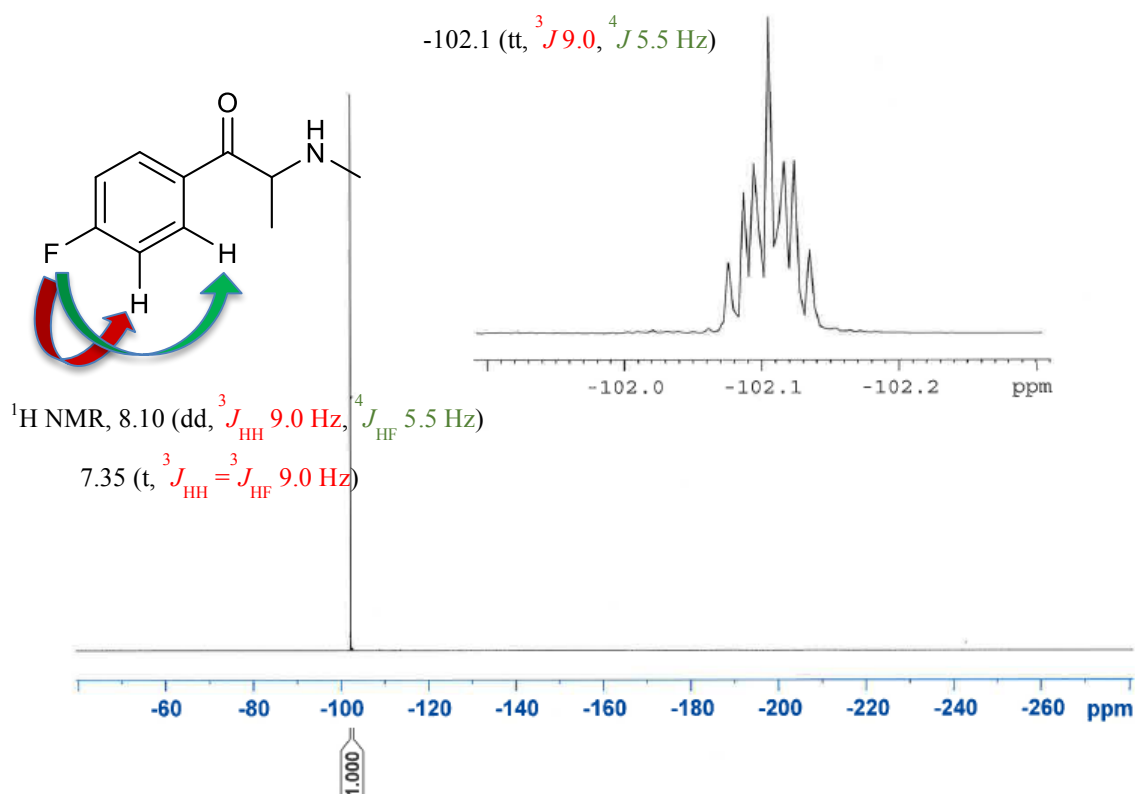


Fig. 5. ^{19}F NMR spectrum (^1H coupled) of flephedrone (D_2O) (in the presence of $\sim 7\%$ benzocaine) with relevant coupling from ^1H NMR also given

4.3.3. *HMBC and ^{15}N HMBC NMR spectroscopy*

The 2D NMR techniques, HSQC (Fig. 6) and HMBC (Fig. 7) were employed for flephedrone and mephedrone, allowing unambiguous assignment of carbons and protons. Focusing on the aromatic signals, the order of the two aromatic peaks in flephedrone HSQC spectrum (Fig. 6) compared to those of mephedrone ^1H NMR were swapped. C3' and C5' are deshielded, due to the high electronegativity of the substituent, fluorine atom. In the mephedrone HMBC spectrum (Fig. 7), C2' and C6' are assigned as more deshielded because of the electron withdrawing effect of carbonyl group (C1). All HMBC correlations of flephedrone and mephedrone (Fig. 8) with 2J and 3J correlations are shown in Table 2. ^{15}N NMR HMBC was recorded to detect the number, kind, and position of the nitrogen atoms proving the presence of a secondary amine coupled with the two *N*- and α -methyl groups (Fig. 9).

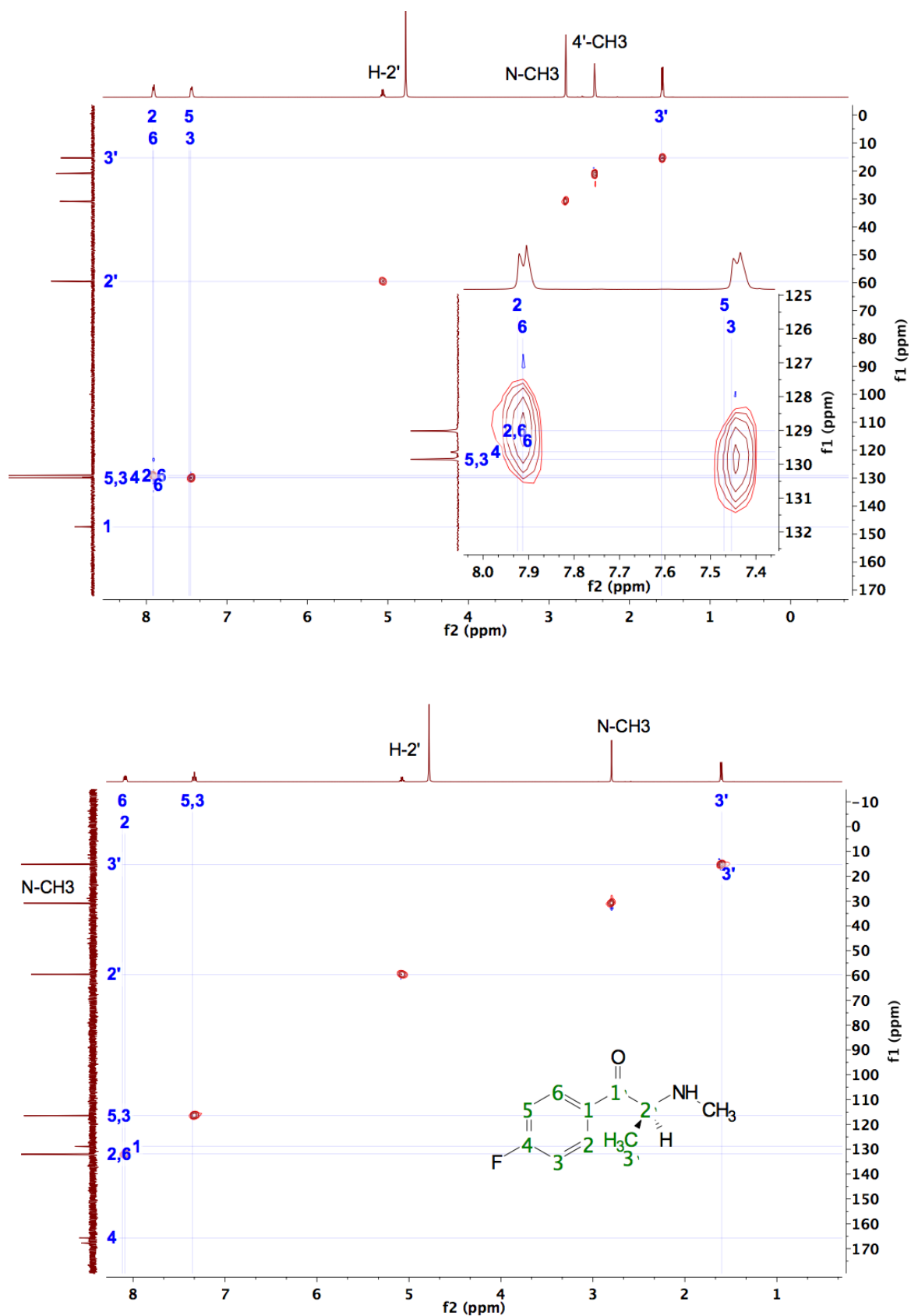


Fig. 6. 2D HSQC NMR of mephedrone (upper) and flephedrone (lower)

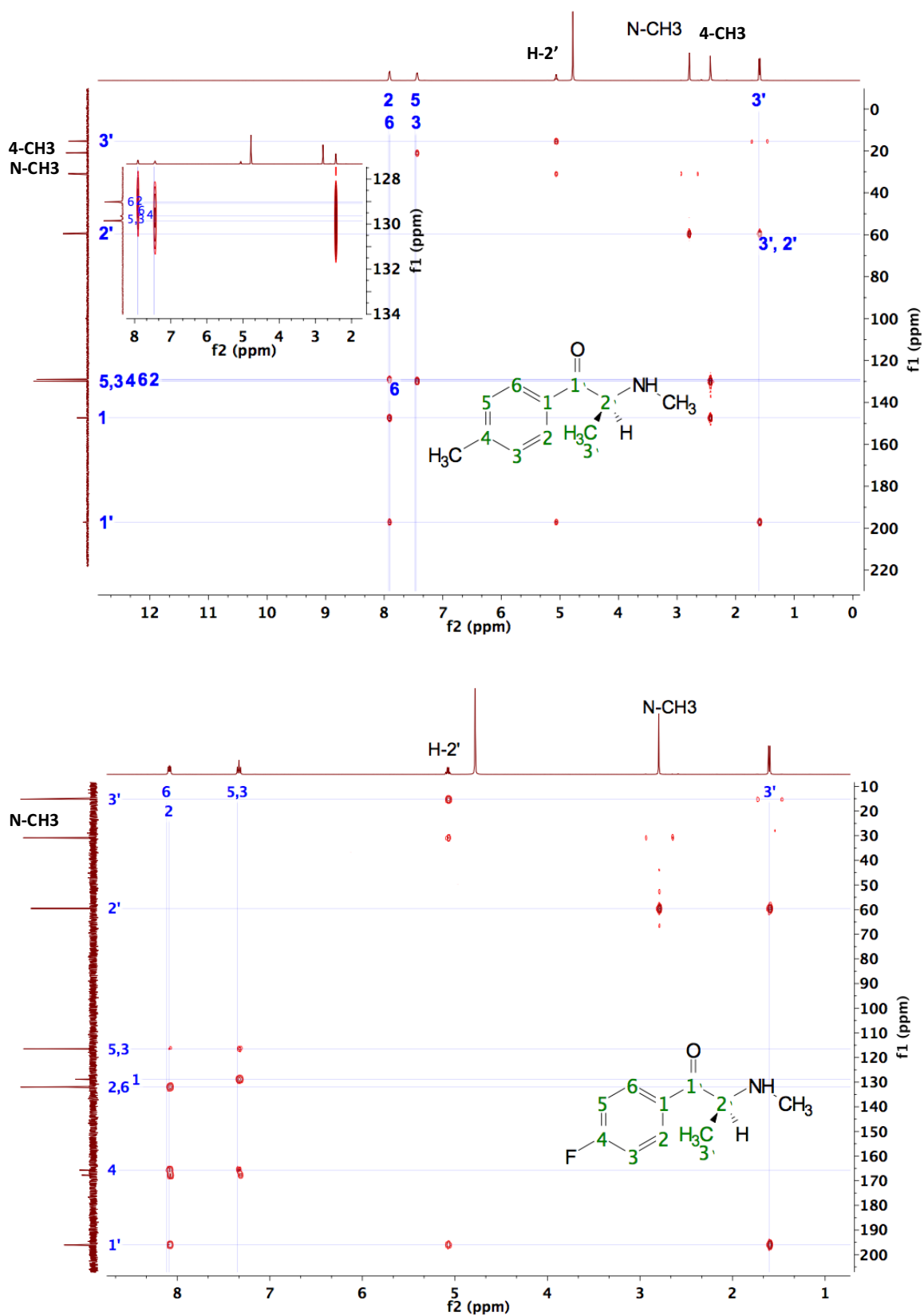


Fig. 7. 2D HMBC NMR of mephedrone (upper) and flephedrone (lower)

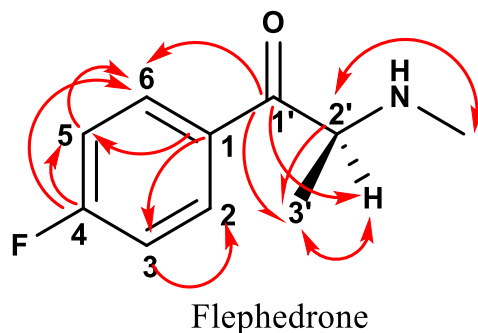
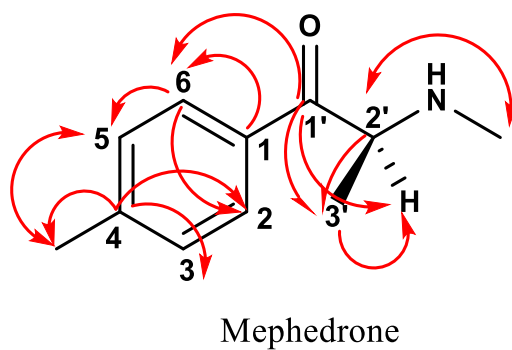


Fig. 8. Key HMBC mephedrone and flephedrone correlations

Table 2

HMBC 2J and 3J correlations of flephedrone and mephedrone (D₂O).

	Flephedrone		Mephedrone	
Position	HMBC		HMBC	
	2J	3J	2J	3J
1		C3, C5	C2, C6	
2, 6	-	C2, C6	C3, C5	C2, C6
3, 5	C2, C6	C3, C5		C3, C5, 4-CH ₃
4	C3, 5	C2, C6	C3, C5, 4-CH ₃	C2, C6
1'		C2, C6, C2', C3'	C2'	C2, C6 - C3'
2'	C3'	N-CH ₃	C3'	N-CH ₃
3'	C2'	-	C2'	-
N-CH ₃	-	C2'	-	C2'
4-CH ₃	-	-	-	C3, 5

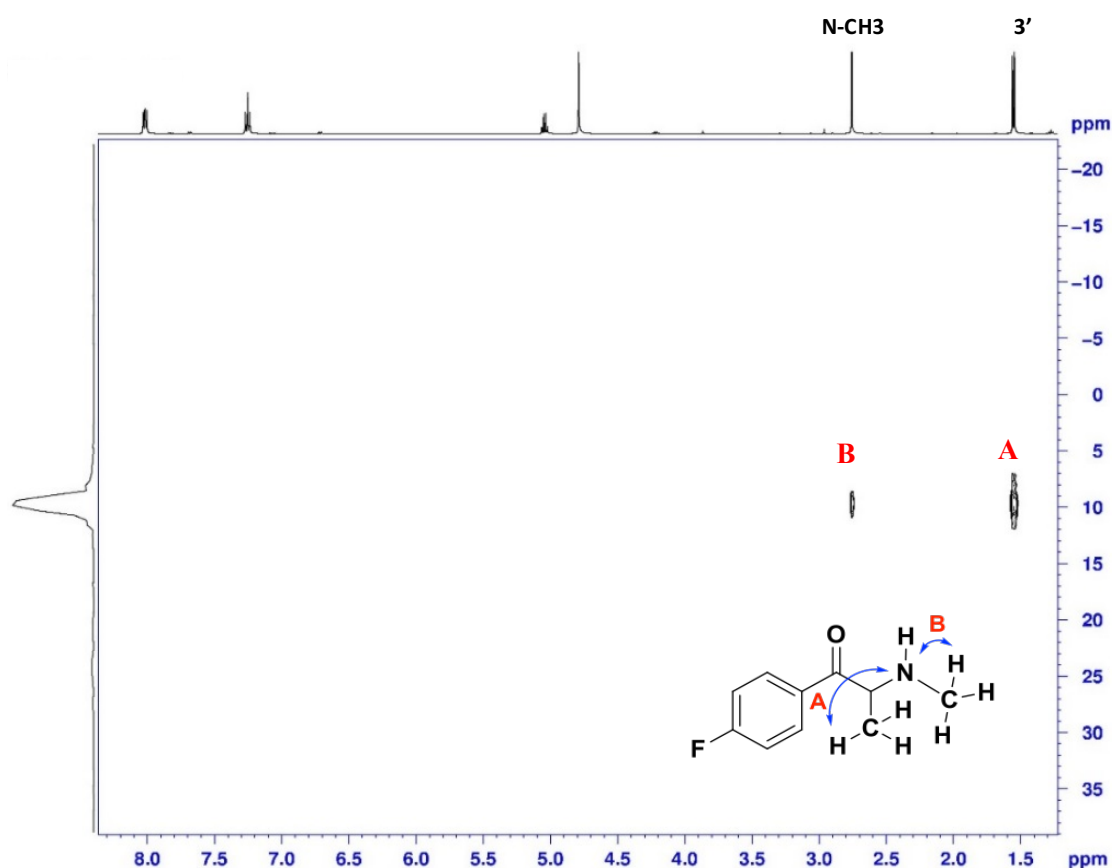


Fig. 9. ^{15}N -HMBC (500 MHz) spectrum of flephedrone (D_2O) showing two ^{15}N - ^1H couplings (with peak assignments)

4.3.4. NOSEY and HOSEY NMR spectroscopy

The classical NOSEY NMR data of both flephedrone and mephedrone were observed (Fig. 10). However, the H-H correlation between 4- CH_3 protons with H-3 and H-5 seen in mephedrone disappeared in flephedrone spectrum, confirming the absence of the *p*-substituted methyl group. Furthermore, the aromatic correlation in NOSEY has no differences between flephedrone and mephedrone (Fig. 10). However, HOESY has been used to assign the interaction between the fluorine atom and the aromatic protons, H-3 and H-5 in flephedrone. The ^{19}F - ^1H HOESY NMR results of flephedrone (Fig. 11) indicate the cross-peaks between the fluorine and H-3 and H-5. These observations are consistent with the ^1H , ^{13}C , and ^{19}F NMR spectroscopic data which confirm the assignment of the aromatic peaks in the NMR of flephedrone.

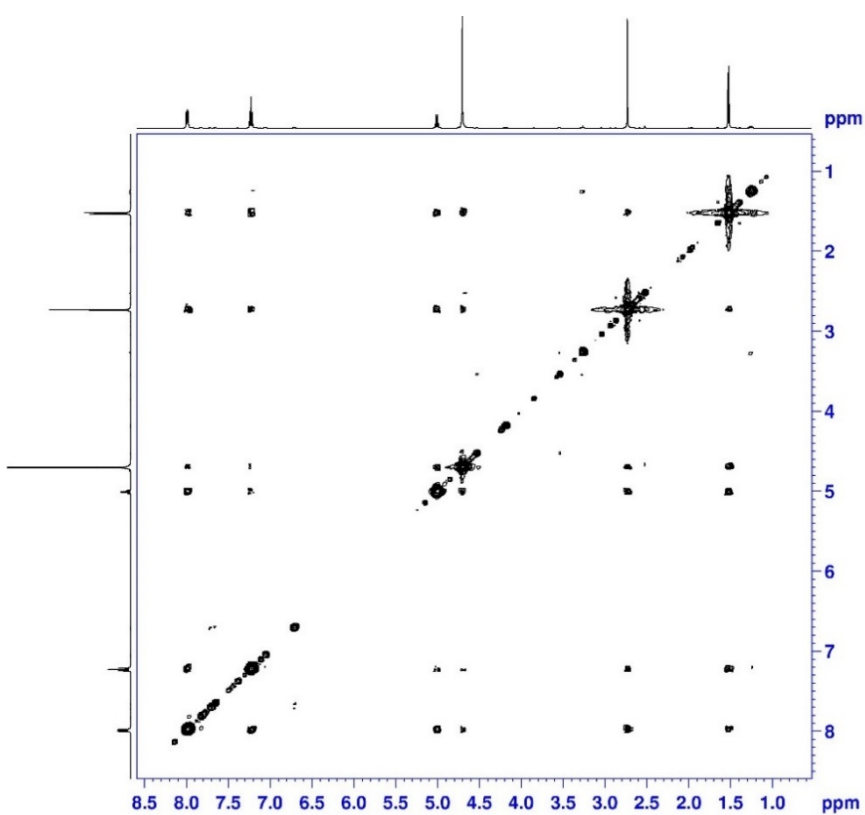
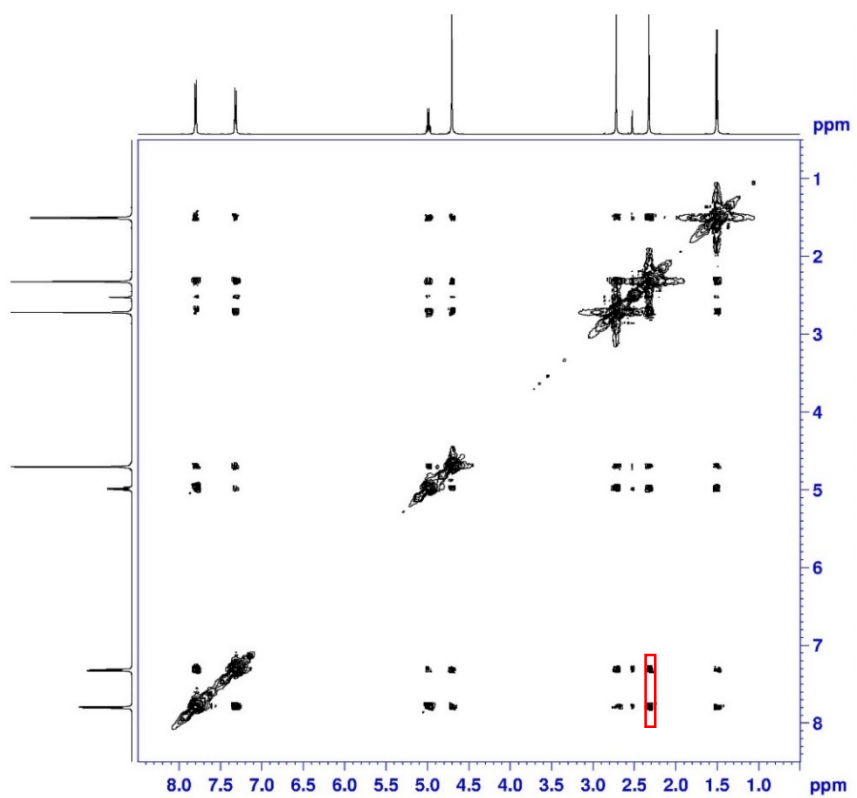


Fig. 10. NOESY NMR (500 MHz) spectrum of mephedrone (upper) and flephedrone (lower) (D₂O)

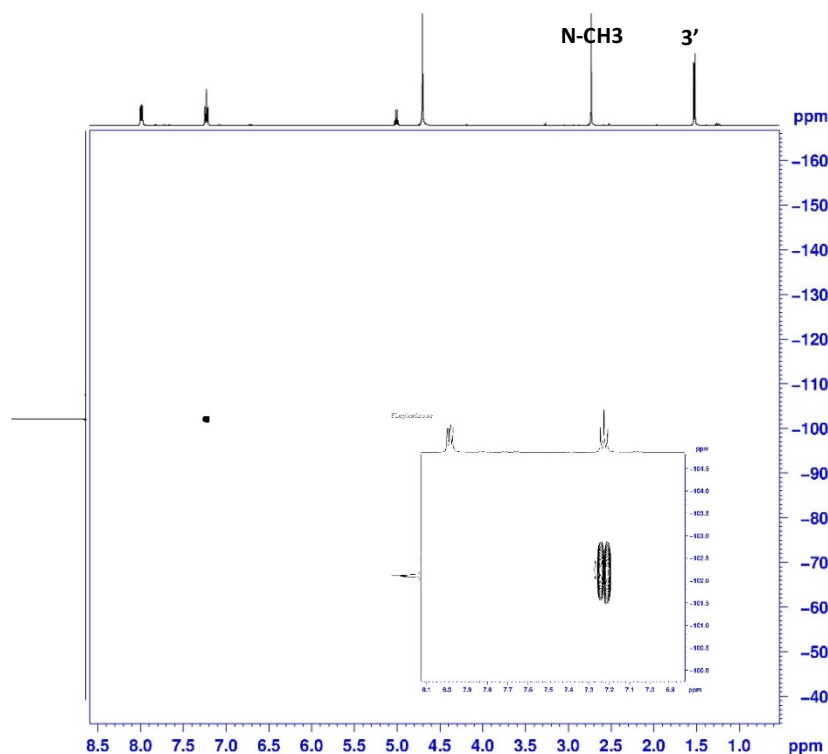


Fig. 11. HOESY NMR (500 MHz) spectrum of flephedrone (D₂O) with the aromatic region expanded

4.3.5. ESI-MS and impurity profiling

The protonated molecular ions $[M+H]^+$ were observed by ESI/QTOF-MS, at 182.0976 and 182.1006 for the 4-FMC samples and the authentic hydrochloride salt of flephedrone respectively, $C_{10}H_{13}FNO$ requires 182.0981 thus confirming the identity of a regioisomer of flephedrone. The sodium adduct $[M+Na]^+$ $C_{10}H_{12}FNONa$ requires 204.0800, found 204.0800. $C_{10}H_{12}FNONa$ ESI-MS spectra also gave $[M+H]^+$ 166.0874. Benzocaine $C_9H_{12}NO_2$ $[M+H]^+$ requires 166.0868; $[M+Na]^+$ $C_9H_{11}NO_2Na$ found 188.0689, requires 188.0687. Both ESI-MS fragments patterns of flephedrone and its cutting agent benzocaine are shown in Fig. 12. The characteristic ions (m/z) of flephedrone were reported by Zuba [10] as 123, 95, and with base peak 58 (m/z). Flephedrone 58 represent $CH_3-CH=NH^+-CH_3$, formed with the flephedrone cation $C_6H_4F-C\equiv O^+$ (m/z 123). Loss of CO from the later gives a 4-fluorobenzoyl cation $C_6H_4F^+$ (m/z 95) [10].

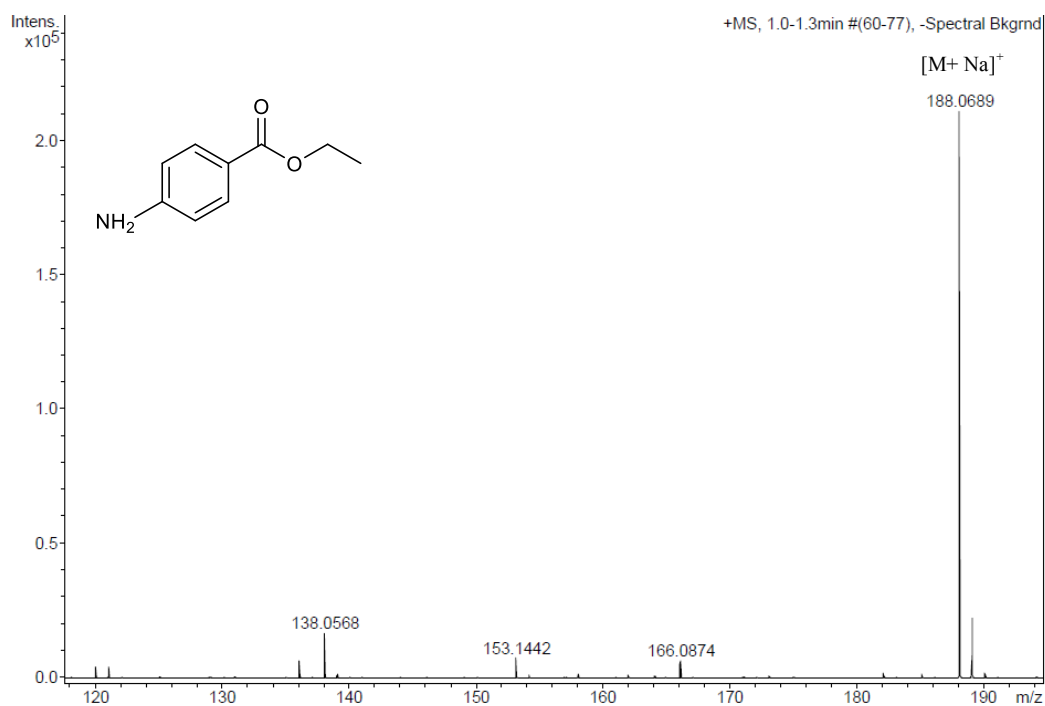
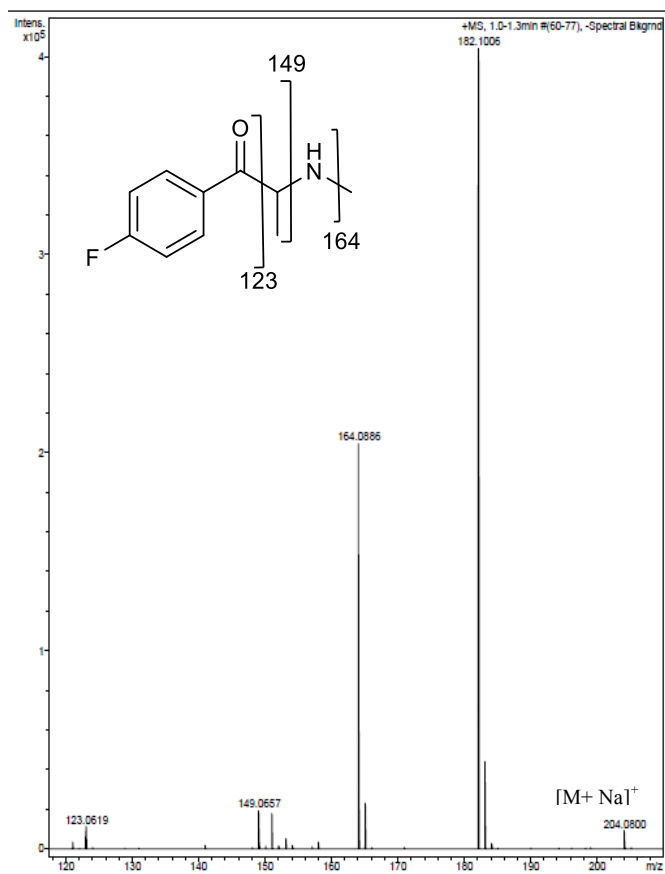


Fig. 12. Fragmentation patterns of flephedrone (upper) obtained by ESI/QTOF-MS and of benzocaine (lower)

4.3.6. Impurity profile of flephedrone samples by NMR and GC-FID

Analysis of the 4-FMC samples by both NMR and ESI-MS showed that they were impure (Figs. 13 and 14). The ^1H and ^{13}C NMR data of the impurity are: ^1H NMR (D_2O): δ 1.36 (t, $^3J_{\text{HH}} = 7.0$ Hz), 4.33 (q, $^3J_{\text{HH}} = 7.0$ Hz), 6.83 (H3 and H5, t, $^3J_{\text{HH}} = 7.6$ Hz), 7.86 (H2 and H6, d, $^3J_{\text{HH}} = 7.6$ Hz) [11]; ^{13}C NMR (D_2O): δ 14.7 (C7), 61.3 (C6), 114.3 (C3, C5), 118.8 (C1), 132.4 (C2, C6), 154.6 (C4), 168.8 (C5) ppm [12]. The NMR data are consistent with benzocaine. A GC–FID method was then developed to resolve flephedrone and benzocaine in mixed samples, achieved with retention times of $R_t = 2.8$ min and 4.9 min respectively (Fig. 15). The benzocaine levels in these 4-FMC samples were quantified by GC (5, 6, 7, and 12%), impurity levels which are in agreement with those determined from the (more approximate) ^1H NMR integration data (4, 6, 7, and 10%). Benzocaine has previously also been quantified by HPLC [13].

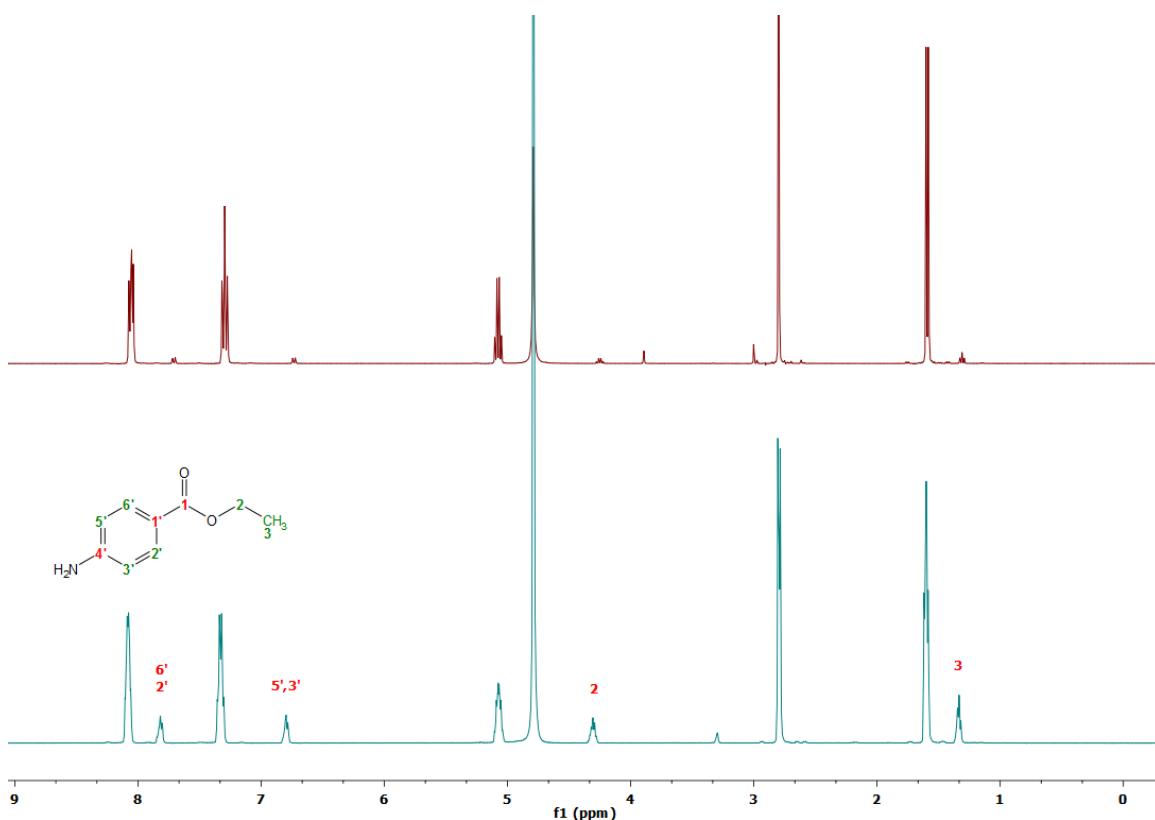


Fig. 13. ^1H NMR (500 MHz) spectrum of flephedrone (D_2O) (seized sample) with the benzocaine ($\sim 7\%$) signals clearly visible (upper) and of authentic flephedrone spiked with benzocaine (lower)

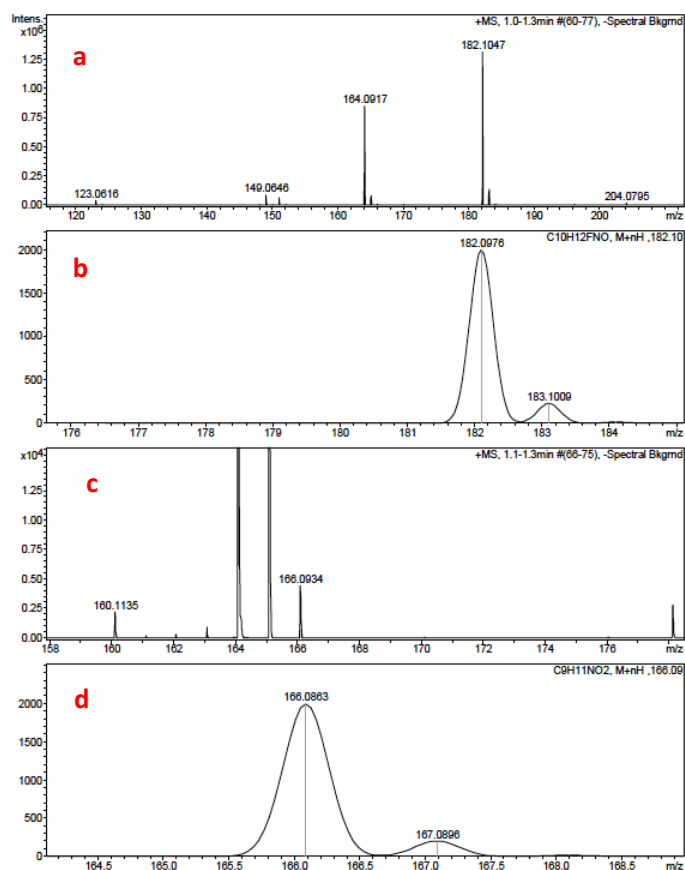


Fig. 14. ESI-MS of (a) flephedrone and (c) benzocaine in a seized sample of flephedrone with their respective MS simulated patterns (b) and (d)

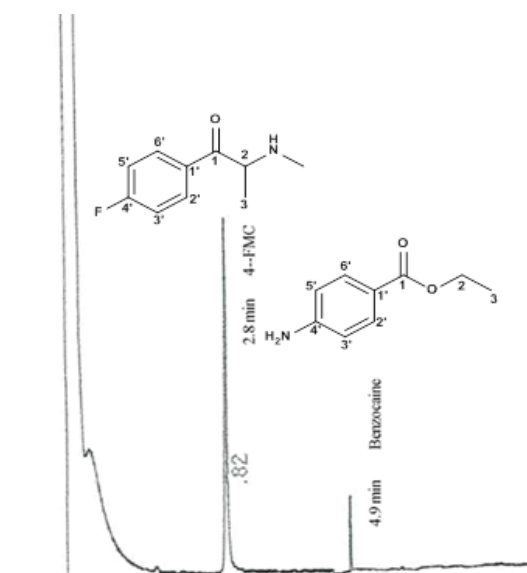


Fig. 15. GC-FID of flephedrone samples impure with benzocaine (showing ring numbering systems)

4.3.7. *Evaluating benchtop ATR-FTIR for profiling flephedrone and its adulterants*

The IR spectra of the street samples of flephedrone were measured using the benchtop ATR-FTIR instrument equipped with the spectra of drugs and “legal highs” database TICTAC. The procedure for using the ATR-FTIR for drugs analysis measurement has few easy steps including; cleaning the crystal surface, measuring the background and then placing a small quantity of a sample on the crystal. After each measurement, the instrument automatically searches to database suggesting likely compounds. A percentage “match” is provided between the sample spectra and the match find in the database (in this case the TICTAC database). Each hit provides a percentage matching between the sample and the library search. If the IR band do not match any signal compounds in the database, e.g. intensity, symmetry and bands width, a Mixture Search should be applied. The number of component is chosen manually, with two or three a good place to start. For example, the ATR-FTIR was used with authentic flephedrone (Fig. 16). The result of the mixture analysis is displayed as a library match with its content % (blue), sample spectrum (“composite spectrum”, maroon), and residual spectrum (green). The residual spectrum indicates the matching level between the library and the compound spectrums, flat line means high similarity. Based on the result, the authentic flephedrone was 100% pure by ATR-FTIR. The software shows details about drug name, form, use, UK control, and provides any alternative name of the drug under investigation.

The same procedures were applied to the street samples of flephedrone, sample codes: AI1, AI2, AI5, and AI16. These were previously analysed by ^1H NMR and all indicated the present of benzocaine in different ratios, 4%, 6%, 7%, and 10% respectively. The results from the ATR-FTIR study showed that all flephedrone and their cutting agent (benzocaine) samples were identified correctly by the TICTAC library search. Atypical result, representative of the mixture analysis of sample AI16 is shown in Fig. 17. The samples were run, 2, 3, and 4 components input into the mixture search. The analysis confirmed the presence of benzocaine, but was not able to identify correctly the ratio of benzocaine, e.g. flephedrone to benzocaine (sample code AI16), suggesting 27.2% benzocaine compared to 10% found by ^1H NMR, 27.2:72.8 benzocaine: flephedrone. The ^1H NMR of AI16 is shown as adulterant compared to 88.1% and 10.5% flephedrone to benzocaine by ^1H NMR integration (Fig. 18). The instrument was not

able to detect impurities $\leq 3\%$ (creatine) while this can be identified in the ^1H NMR at 1.31%. referenced to the satellite peaks.

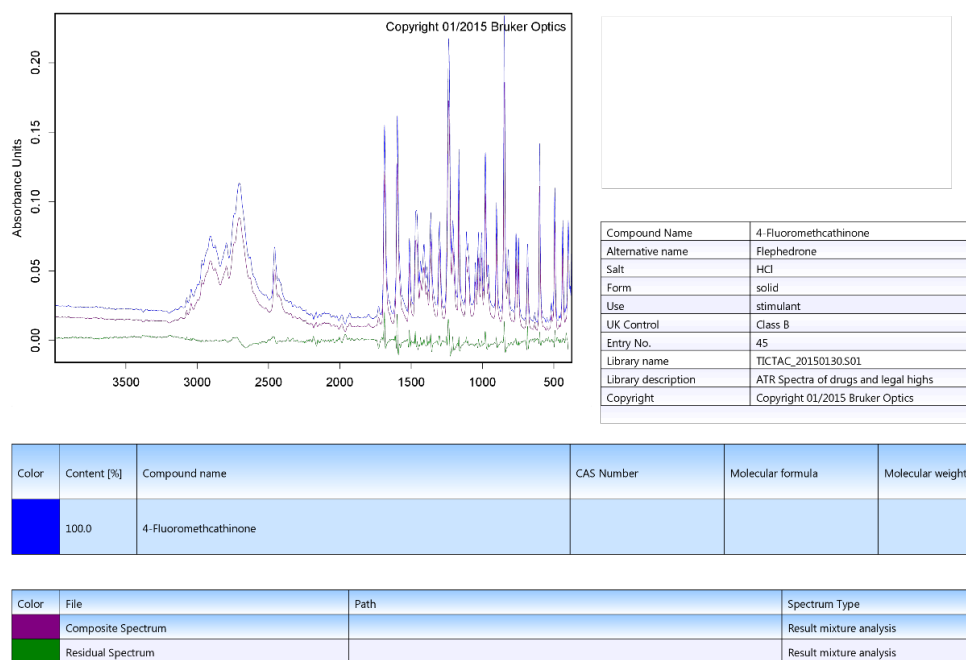


Fig. 16. IR of an authentic flephedrone and results of the TICTAC library search match

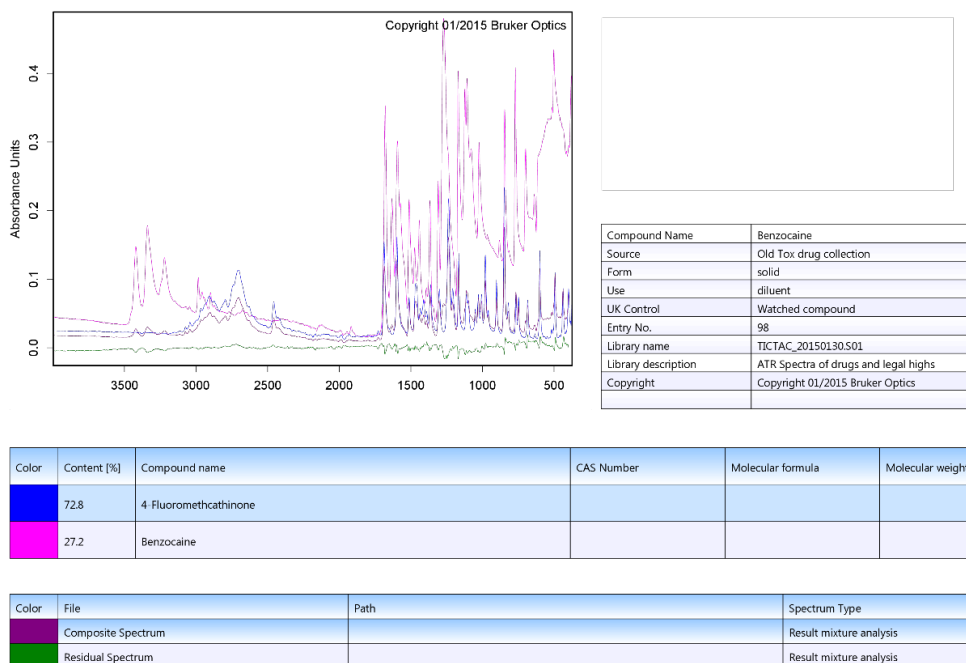


Fig. 17. Street sample of flephedrone (AI16) cut with benzocaine and their matching spectrum results from the TICTAC library

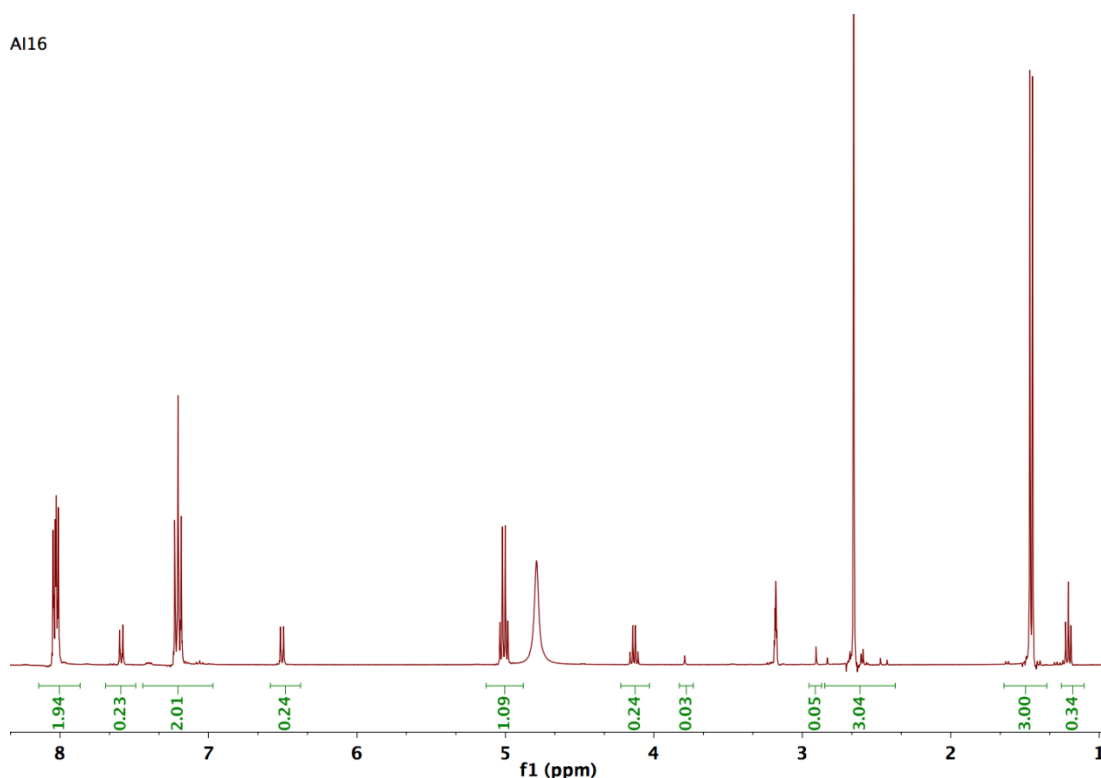


Fig. 18. Confirming the presence of flephedrone and benzocaine in AI16 as well as traces of creatine

4.4. Conclusions

4-FMC is a designed cathinone derivative either designed to side-step the banning of the “legal highs” 4-MMC and/or with one eye on the potential pharmacological enhancement from incorporating a fluorine substituent in a biologically active small molecule as “there is also evidence that single, carbon-bound fluorine substituents, particularly when on an aromatic ring, can exhibit specific polarity influences, including H-bonding, that can strongly influence binding” [1]. The human toxicology of flephedrone is not well established [14]. Lipophilicity is significant as it often controls the absorption, transport, or receptor binding of a compound and so can enhance its bioavailability. The effect on the lipophilicity of fluorine substituents on an aromatic ring was calculated by Dolbier showing that CF₃ ($\pi_x = 0.88$) is greater than CH₃ ($\pi_x = 0.56$) following from the respective *n*-octanol/water partition coefficients [1]. So, perhaps counter-intuitively, the presence of the most powerful electronegative atom, a fluorine substituent, gives rise to enhanced lipophilicity.

Benzocaine is a well-known topical anaesthetic. However, the FDA has reported that using a gel or spray containing benzocaine could cause a serious blood disorder, methemoglobinemia [15, 16]. Similar concerns are raised elsewhere, e.g. Taleb and co-workers review the evidence where, even following topical application of benzocaine, oxidized haemoglobin might increase the chance of methemoglobinemia [17]. Recently, a case of acquired methemoglobinemia due to the frequent use of the legal high “Pink Panthers” was reported in parallel with the increasing prevalence of “legal highs” particularly those containing added benzocaine [18].

Benzocaine is currently being used in the illicit drugs market as a common adulterant (cutting agent) to increase profit [19]. Indeed, it is now commonly found in cocaine HCl [19-21], which might link the supply of such adulterated flephedrone to that of other commonly cut illicit drugs. Drug dealers may use benzocaine as it is easily accessible and similar in physical appearance to flephedrone. In sufficient quantity, benzocaine might be added to flephedrone in order to mimic the initial numbing (anaesthetic) effect of cocaine [22] and possibly even to sell the mixture, not as flephedrone, but as cocaine.

Our rapid analysis and quantification of 4-FMC and benzocaine compares well with the literature methods [7, 13]. After our analyses were complete, Kerrigan and co-workers reported [23] on the thermal degradation of synthetic cathinones including flephedrone and the implications of this for forensic toxicology. They stated that such thermal degradation had received little attention. In situ thermal degradation during GC–MS analysis was by oxidative degradation, by the loss of two hydrogens, yielding a characteristic 2 Da mass shift. Such degradation products were characterized by prominent iminium base peaks with mass-to-charge (m/z) ratios 2 Da lower than the parent drug. As the degradation was thermal, it was minimized by lowering injection temperatures and/or residence time in the inlet during GC analysis [23]. Research is also on-going to replace aspects of wet-chemistry based quantitative analysis on minimal trace amounts of illegal drugs with rapid in situ, e.g. potentially on-site analysis in a nightclub, screening using handheld (therefore portable) spectrophotometers [24, 25]. The use of Raman spectroscopy to detect counterfeit medicines [24] may be extended to the analysis of street drugs, whilst discrimination between simple drug mixtures has been achieved using a deep ultraviolet-visible (DUV-Vis) reflected optical fibre sensor [25]. Both techniques can detect and possibly quantify known drugs for which standard spectra are available, but they are not suitable for the analysis of new drugs.

The combination of ^1H , ^{13}C , ^{15}N , and ^{19}F NMR spectroscopy was used to identify and decipher between flephedrone regioisomers and other cathinones such as mephedrone. 2D NMR techniques, ^{15}N HMBC and HOSEY were reported for flephedrone and mephedrone, allowing unambiguous assignment of the aromatic regions. We have separated flephedrone and benzocaine with base-line resolution by GC-FID and quantified the amount of benzocaine adulterant in four (nightclub) flephedrone samples by GC, the ^1H NMR integration was in broad agreement. The street samples of flephedrone were also analysed by ATR-FTIR and compared with ^1H NMR. ATR provides an excellent fast scanning technique for street flephedrone samples with no sample preparation needed. However, ^1H NMR proved to be more powerful and accurate with regard to impurity profiling analysis. These data show that this now illegal cathinone-derived stimulant (a high) is now being cut by 5-12% with benzocaine, so drug users cannot be certain of the purity of the drug they are taking. Indeed, another recent paper, published this month, on the diffusion of NPS in the illicit drugs market (2013-15) into Italy, shows that flephedrone is a problem for public health in Italy [26]. Benzocaine as an adulterant can cause serious health problems, potentially more harmful than flephedrone. Therefore, there are risks from the pharmaceutically active cutting agents themselves.

4.5. References

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Chapter 5

Impurity profiling of seized and amnesty bin mephedrone samples

5.1. Introduction

The worldwide emergence of cathinones as new psychoactive substances (NPS) was recently underlined in the Early Warning Advisory on New Psychoactive Substances UNODC [1]. Mephedrone (4-methylmethcathinone, 4-MMC) is a synthetic cathinone, a β -keto phenethylamine, derived from cathinone which naturally occurs in Khat. The first cathinone derived NPS were controlled as single compounds and cathinone derivatives were synthesised to avoid the law by adding substituents at different positions of the cathinone molecule (Fig. 1) [2]. Synthetic cathinones are claimed to have both sympathomimetic and stimulation effects with easy access to the brain across the blood-brain barrier [3] producing a psychoactive amphetamine- or cocaine-like stimulant effects [4]. Mephedrone is reported to have undesirable side effects including, but not limited to: anxiety, high blood pressure, nosebleeds after snorting, insomnia, hallucinations, and abdominal pain, hospitalisations are also reported [5]. Dependence and tolerance are reported in mephedrone abusers [6].

Mephedrone has no history of medical use. This drug is abused for its stimulants and euphoric effects [7]. “Meow/Miaow”, “Bubbles”, “Crab”, “Blow” are some of the many street names of mephedrone [8]. Mephedrone was synthesised in 1929 and first appeared on the market as an illicit drug in 2007 in part due to the shortage of MDMA [9]. After that, the popularity of mephedrone rose in Europe, especially over the internet, to become one of the most popular drugs in the last decade [7].

In the UK, mephedrone was recently controlled under the Misuse of Drugs Act as a Class B drug in April, 2010. Despite the control, mephedrone abuse has been increasing in England [10]. Deaths related to the consumption of mephedrone have been reported [5]. These have increased from 1.0% (n = 22) to 1.5% (n = 34) between 2014 and 2015. While deaths involving MDMA use increased from 1.1% (n = 25) in 2014 to 1.2% (n = 28) of total cases in 2015. The majority of these deaths

are related to mixing mephedrone with other drugs such as cocaine, amphetamine, methamphetamine, heroin, γ -hydroxybutyrate, and MDMA [11].

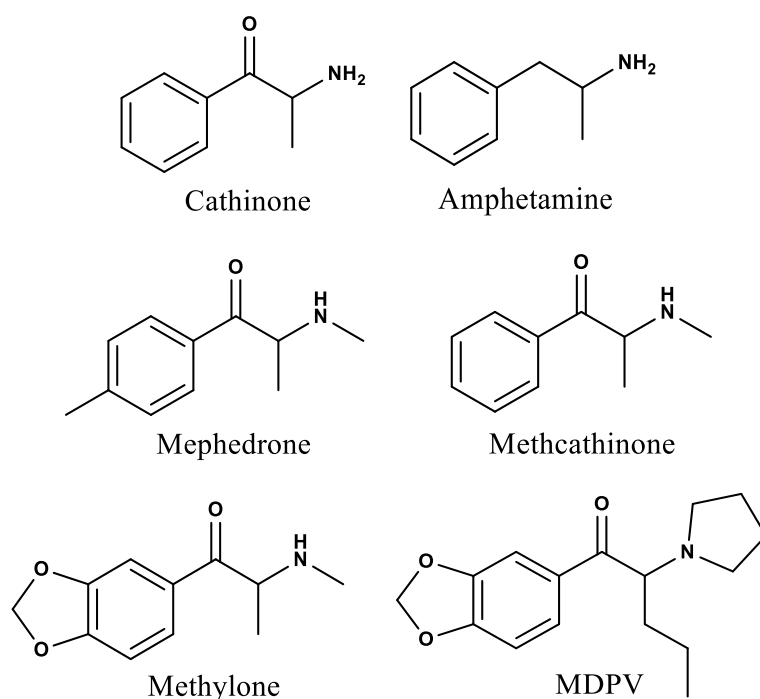


Fig. 1. Cathinone and its structural similarity to amphetamine and related cathinone derivatives

A wide variety of quick and simple tests have been used for mephedrone analysis including Zimmerman colour test [12], a high sensitive microcrystal test [13], Raman and infra-red IR [14]. The later two techniques have been used for illicit drug analysis, (as non-destructive techniques) though both methods need statistical analysis to interpret the data [15]. Chromatographic technique, gas-chromatography mass-spectrometry (GC-MS) [16], GC-FID, and liquid chromatography with high resolution mass spectrometry-mass spectrometry (LC-MS/MS) have been widely used for detecting mephedrone [17, 18, 19]. GC-MS is considered an established method for illicit drugs analysis in forensic science labs by providing a chromatographic separation and mass identity of the drugs [20]. However, MS cannot differentiate between isomeric structures with high degree of certainty [21] and reference standard material is needed to confirm each drug's identity [12]. On the other hand, NMR spectroscopy allows exactly this kind of differentiation between cathinone regioisomers with no need for reference standards

[18, 19, 22]. Furthermore, in NMR spectroscopy, there is no need to test a detections response factors, while this is required for each instrument in other spectroscopic and spectrometric quantitative methods [23].

NMR spectroscopy has a wide history of use in illicit drug profiling [24] and impurity/purity profiling [25]. It has also been used to determine geographic origin by Site-specific natural isotopic fractionation (SNIF) measurement [26]. The capability of NMR for quantitative measurement as a “primary” method was discussed by Holzgrabe et al. in 2005 [27]. Quantitative analysis by NMR (qNMR) has consequently been used for quality control and drug discovery [27, 28], natural products [23], excipients [29], and in food production [30]. A comprehensive study by Yang et al. compared the qNMR technique to HPLC [31]. One limitation of qNMR is overlapping peaks, however this problem can be tackled as all protons across the spectrum are equally sensitive [23]. Despite the lower sensitivity level of NMR compared to mass spectrometry, quantitative analysis by NMR does have an advantage in that it does not require reference materials [23]. Indeed, there have been noticeable developments in modern qNMR spectroscopic technique leading to sensitivity enhancement.

Regarding impurity determination of mephedrone samples, the first purity study was conducted by Singh in 2010 [32]. The analysis performed on samples were bought from the Internet and the mean purity reported was 80%. However, the purity was not accurate because no reference standard of mephedrone was available and samples with high purity were considered as 100% pure [32]. Two samples with street names “Recharge” and “Doves Original” were marketed as bath salts and plant food containing mephedrone at 42% and 12%, respectively [33]. Another study in 2014 by Miserez et al. investigated mephedrone purity in 119 samples that had been collected in an amnesty bin from South Wales in the UK between November 2011 to March 2013, a year after the banning of mephedrone. The purity was determined by GC-MS and the adulterants were profiled by FT-IR. In this study, the purity dropped from 80% at the beginning of the analysis to 50% for the last 10 samples [34].

In this chapter work using different analytical techniques will be described. In particular, the use of NMR and MS for mephedrone purity/impurity profiling with the implementation of quantitative NMR (qNMR) to identify and quantify mephedrone in street samples. Diffusion-Ordered Spectroscopy (DOSY) NMR is tested as well to see how practical it is for impurity identification and separation.

The impurity assessments were cross-validated by applying statistical analysis; PCA, scree plots and dendrogram plots on the NMR data. These were applied to classify the samples based on their similarity and/or differences to extract information to aid law enforcement in Avon Somerset (UK), from where the samples were provided.

5.2. Experimental

5.2.1. Materials

Authentic mephedrone, creatine monohydrate ($\geq 98\%$), benzocaine B.P. and L-glutamic acid monosodium salt hydrate (MSG, $\geq 99\%$, TLC), deuterium oxide, D₂O and maleic acid as a standard for quantitative NMR, TraceCERT[®] were purchased from Sigma-Aldrich, UK. Sodium 3-trimethylsilylpropionate, TMSP-2, 2, 3, 3-D₄ (D, 98%) was purchased from Cambridge Isotope Laboratories, Inc., USA. Caffeine (98.5%, USP/BP) was purchased from ACROS Organics[™], USA. All other solvents were of HPLC grade and purchased from Fisher or VWR, UK.

5.2.2. NMR analysis

5.2.2.1 Sample preparation and data processing

¹H, ¹³C, HSQC, HMBC, COSY, DOSY, qNMR and DEPT NMR spectra were recorded on a 500 MHz Bruker Spectrometer. Samples for impurity profiling were powdered and 30 mg were dissolved in 0.6 mL D₂O. To quantify mephedrone in the street samples of mephedrone a qNMR experiment was performed. An internal standard (IS) of maleic acid (1 mg/mL, 1.0 mM) was prepared in D₂O containing 0.25 % TSP for 0 ppm reference. Each sample was ground and accurately weighed, 10 mg then vortexed in the D₂O mixture until the sample dissolved and made up in a 5 mL volumetric flask. Any sample with insoluble materials was gently warmed before the qNMR analysis to ensure all material was dissolved. Maleic acid has been chosen as internal standard as it is soluble in water, chemically stable, has one peak in D₂O at 6.2 ppm which does not overlap with the mephedrone or impurity signals. The IS (maleic acid, 1 mg/mL in D₂O) was quantified (qNMR) four times to test the precision of the instrument. The reproducibility of the method was evaluated

by measuring (in triplicate) the different weights of a few samples chosen at random. All spectra were manually baseline-corrected and processed by using MestReNova (Version 10.0.2) and Bruker TopSpin software for the NMR data processing. The analytical balance was a Sartorius Microbalance SE-2F, precision 0.1 µg; Sartorius AG, Goettingen, Germany.

5.2.3. Mass Spectrometry

The High Resolution Time-of-Flight (TOF) mass spectra were obtained on a Bruker Daltonics “microTOF” mass spectrometer using electrospray ionisation (ESI) (Bruker Daltonik GmbH, Bremen, Germany). The positive $[M+H]^+$ and negative $[M-H]^-$ mode of MS-ESI data of mephedrone seized samples and their impurities were measured. The mephedrone samples for the ESI-MS measurement were prepared in 10 µg/mL concentration in methanol. The mephedrone and their impurities peaks were extracted by using the data Analysis software version 4.3 (Bruker Daltonik GmbH, Bremen, Germany).

5.2.4. Chemometric analysis of the 1H NMR

The 1D 1H NMR spectra of the 21 mephedrone samples were processed by MNOVA. All samples were referenced to TSP (0.0 ppm), baseline corrected and normalized to the sum values of the signals excluding the solvent and the IS peaks. After that each spectrum was sliced into 0.04 ppm parts between 0 and 9 ppm and data exported as comma/colon separated values (CSV) format to MestReNova (Version 10.0.2) and R statistic software. Different statistics tests were applied on 225 variables for each spectrum: PCA, scree plot and dendrogram.

5.3. Results and discussion

5.3.1 Chemical profiling of mephedrone samples and their impurity

5.3.1.1. ^1H NMR and Mass Spectrometry results

21 samples of mephedrone from South West police seizures (7 samples, 2013 and 2015), a Bristol night club (12 samples, 2013) and the 2013 Glastonbury music festival (2 samples, 2013) were obtained from the Drug Expert Action Team (DEAT), Avon and Somerset Constabulary, UK. Mephedrone street samples ranged from creamy needles to dark brown sticky powder/crystals (Fig. 2). 1D and 2D NMR assignments of mephedrone were discussed previously in chapter 3.

Mephedrone can be mixed with drinks, sniffed, injected and smoked. Impurity profiling can provide valuable information related to a method of administration. Mixed routes are also reported to be used within a drug taking session: oral and nasal, oral and rectal [7], e.g. samples cut with sucrose are more likely to be mixed with a drink or sniffed, because if this mixture is smoked, it will burn. Adulterates are deliberately added either as bulking agents, e.g. sugar, or to enhance the drug's effect, e.g. caffeine as a stimulant. Topical anaesthetics, e.g. benzocaine, lidocaine are reported to be used as drug adulterants to mimic the effects of cocaine and might even be sold as cocaine [35]. In 2013, over than 2 tonnes of benzocaine, lidocaine, and phenacetin were seized at the border [35]. Samples with the same cutting agent in the same ratios might come from same drug dealer. While if the mephedrone is pure, it might be possible to link back to a manufacturer.



Fig. 2. Mephedrone street samples illustrating different form and colour

The data from the ^1H NMR illustrates that only 11% of the mephedrone samples were pure (Fig. 3) and this shows how legal control has affected mephedrone purity. Reduction in drugs purity can be dangerous in terms of overdoses, where users cannot be certain about the correct dose [34]. In this analysis all signals in mephedrone samples were identified and assigned, then confirmed by comparing these with an authentic mephedrone sample. Mephedrone samples were found to be cut with MDMA, MSG, creatine, benzocaine (BZ), and sucrose (Fig. 4). Both MDMA and mephedrone share the same physical effects [36] and this might be the reason why drugs dealer cut mephedrone with MDMA. A list of the cutting agents identified within the street samples of mephedrone with their ^1H NMR chemical shifts are given in Table 1. A list of the positive and negative MS values of the impurities identified within the mephedrone samples are given in Table 2.

The most frequent cutting agents were MSG and creatine with 14% and 11% respectively (Fig. 3). This is due to the availability and the crystal forms of these are almost identical to mephedrone crystal shape. The ^1H NMR assignment of creatine monohydrate are shown in Fig. 5. The samples and their impurities are also confirmed by ESI-MS. The ESI-MS analysis for mephedrone cut with creatine confirm the presence of mephedrone and creatine with an $[\text{M}+\text{H}]^+$ peak at 178.1239, $\text{C}_{11}\text{H}_{16}\text{NO}$ requires 178.1226 (7.2 ppm); $[\text{M}+\text{Na}]^+$ $\text{C}_{11}\text{H}_{15}\text{NONa}$ requires 200.1046, found 200.1046 confirming the identity of mephedrone Fig. 6. For the creatine monohydrate, the $[\text{M}+\text{H}]^+$ $\text{C}_4\text{H}_{10}\text{N}_3\text{O}_2$ requires 132.0767, found 132.0768; $[\text{M}+\text{Na}]^+$ $\text{C}_4\text{H}_9\text{N}_3\text{O}_2\text{Na}$ requires 154.0587, found 154.0597; $[\text{M}-\text{H}]^-$ $\text{C}_4\text{H}_8\text{N}_3\text{O}_2$ requires 130.0622, found 130.0635. The extracted MS spectrum of a mephedrone cut with creatine is illustrated in Fig. 6. The MSG was difficult to detect by ESI-MS as this molecule can be converted by cyclization to pyroglutamate under acidic, alkaline, and/or high temperature conditions [37]. However, MSG can be characterized clearly by NMR, see Fig. 7.

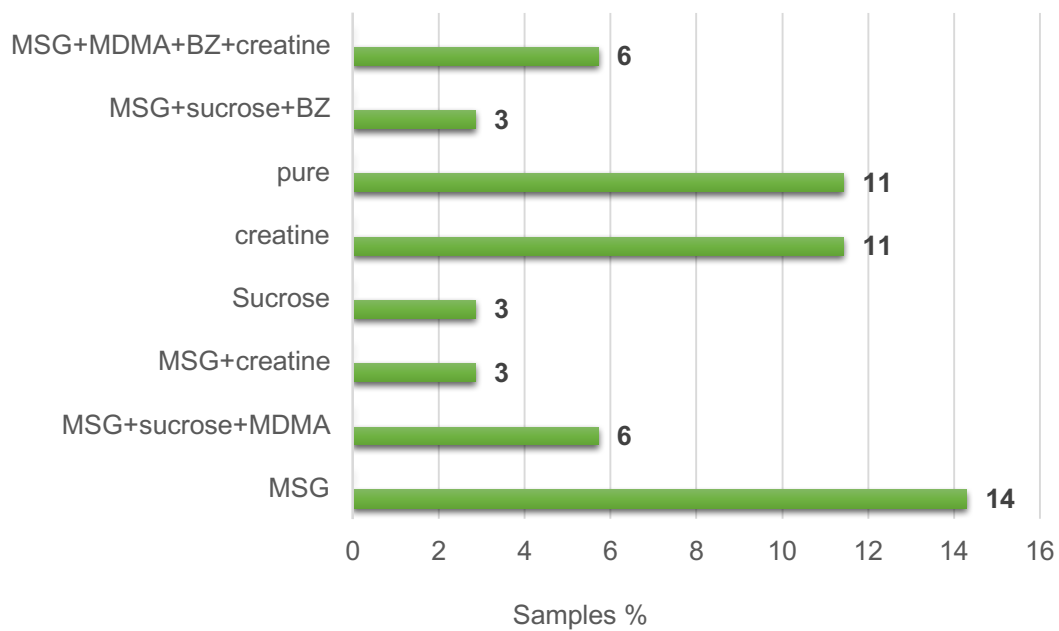


Fig. 3. Frequency (percentage) each cutting agent was detected in samples of mephedrone

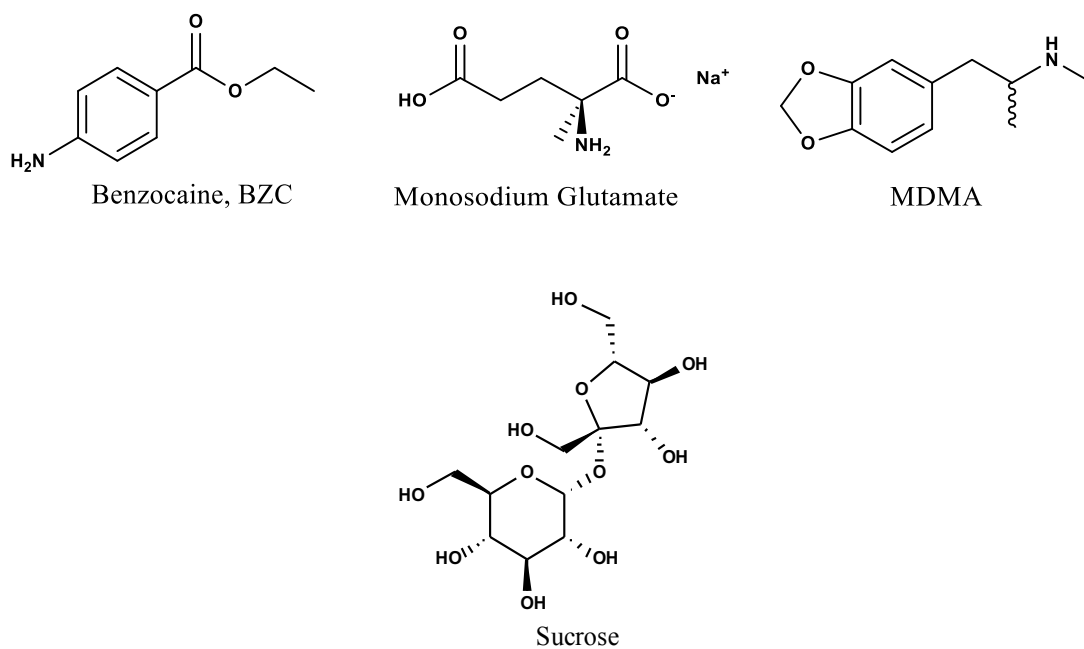


Fig. 4. Cutting agents identified in street samples of mephedrone

Table 1

Characteristic chemical shifts in ^1H NMR of mephedrone and impurities/cutting agents in D_2O

Impurity	^1H NMR chemical shift of the impurities in D_2O
Mephedrone	1.50 (CH_3), 2.30 (CH_3), 2.70 (CH_3), 4.90 (CH), 7.30 (CH_2), 7.80 (CH_2) ppm
MDMA	1.26 (d, CH_3), 2.68 (s), 2.83 (m), 3.46 (m), 5.60 (s), 6.78 (t), 6.82 (s), 6.87(d)
Benzocaine	1.33 (t), 4.29 (q), 7.26 (d), 7.75 (d)
MSG	2.08 (m, CH_3), 2.35 (t, CH_3), 3.75 (t, CH)
Sucrose	5.41 (s, CH)
Creatine	3.00 (s, CH_3), 3.90 (s, CH_2)

Table 2

The positive and negative MS values of the impurities identified in the mephedrone samples

	ESI-MS Positive mode				ESI-MS Negative mode	
	Found		Requires		Found	Requires
Impurity	$[\text{M}+\text{H}]^+$	$[\text{M}+\text{Na}]^+$	$[\text{M}+\text{H}]^+$	$[\text{M}+\text{Na}]^+$	$[\text{M}-\text{H}]^-$	$[\text{M}-\text{H}]^-$
MDMA	194.1219	-	194.118	216.1000	-	-
Benzocaine	166.0874	188.0689	166.0868	188.0687	-	-
Sucrose	-	-	-	-	341.1106	341.1089
Creatine	132.0781	154.0597	132.0767	154.0587	130.0635	130.0622

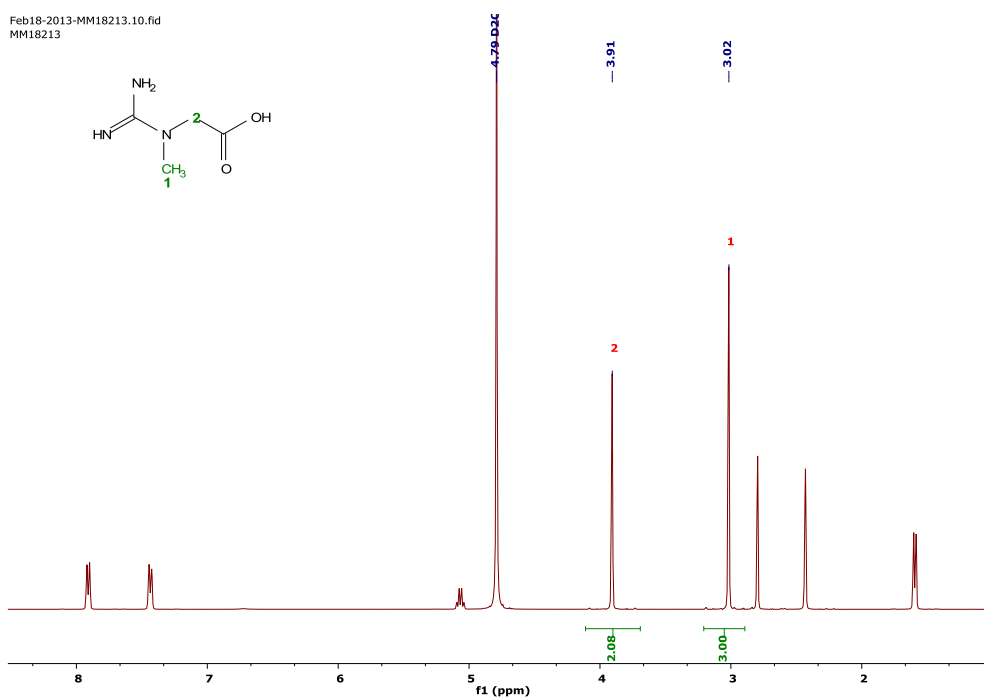


Fig. 5. ^1H NMR spectrum of mephedrone cut with creatine monohydrate (peaks for creatine are assigned)

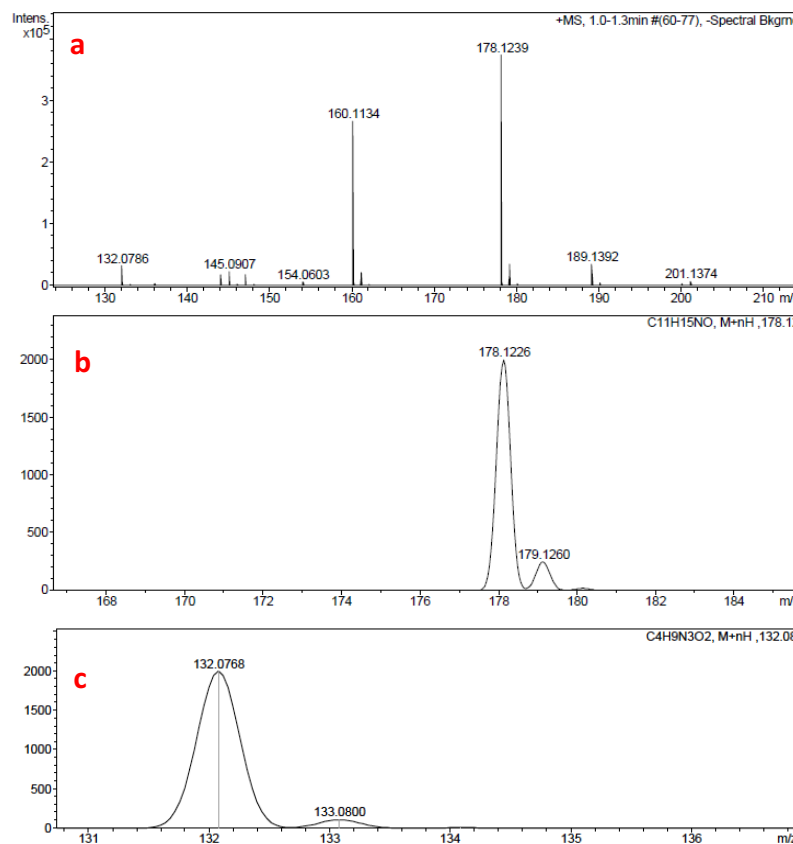


Fig. 6. Mass spectra of (a) mephedrone cut with creatine monohydrate with (b) the exact mass ion of mephedrone, and (c) simulated pattern for creatine

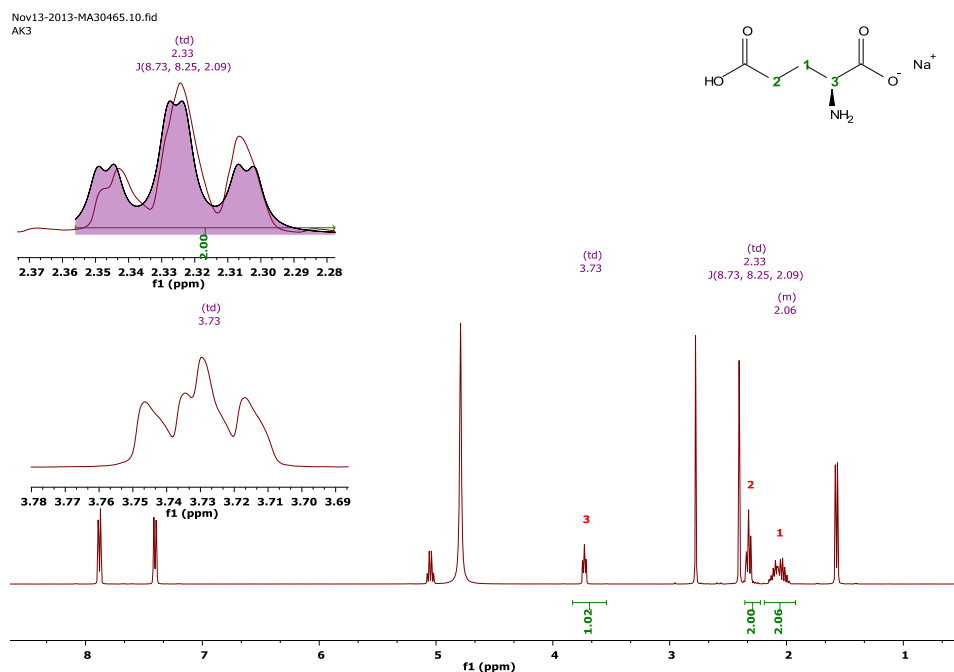


Fig. 7. ^1H NMR assignment of mephedrone cut with MSG in D_2O , the structure of MSG is also shown with the 2.33 and 3.73 ppm peaks expansion

Both MSG and creatine are likely to have been chosen by drug dealers as cheap, safe and readily available cutting agents [34]. In this case mephedrone with these fillers can be smoked, or mixed with drink but not sniffed because creatine will convert into a gel in the nose while MSG is a salt and harsh in a nose if it is sniffed. Sucrose as a safe filler choice is also used, 3% of the mephedrone samples were cut with sucrose (Fig. 3). MDMA was identified in four mephedrone samples that also contained further cutting agents. The first two mixtures contained mephedrone, MDMA, MSG and sucrose in very similar ratios, 23:50:16:0.5 and 23:44:17:0.4 for AM7-1 and AM7-2 respectively (Fig. 8, AM7-1 and AM7-2). The high similarity in impurities including their ratios, might suggest these come from same source. The second mixtures contained: MSG, benzocaine, and sucrose in different ratios, (Fig. 9, AQ14-1), and the third one contain MDMA, creatine, benzocaine, sucrose, and MSG (Fig. 10, AI6-1). These impurities were confirmed by comparing the spectrum with authentic samples of the adulterants. The impurity profiling of AQ14-1, and AI6-1 (Fig. 9 and Fig. 10) indicate that these two samples were initially cut with benzocaine, creatine, or/and MDMA. While the large amount of MSG adulterant might be as a result of a further cut by a second dealer.

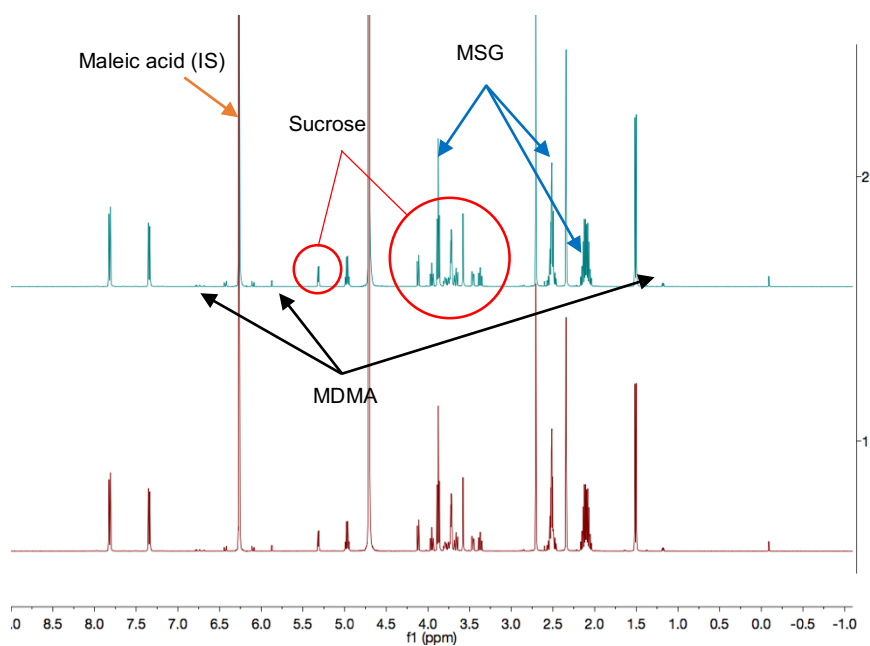


Fig. 8. Stacked spectrum of mephedrone, AM7-1 (1) and AM7-2 (2) cut with the same mixtures and similar ratios of MSG, MDMA and sucrose

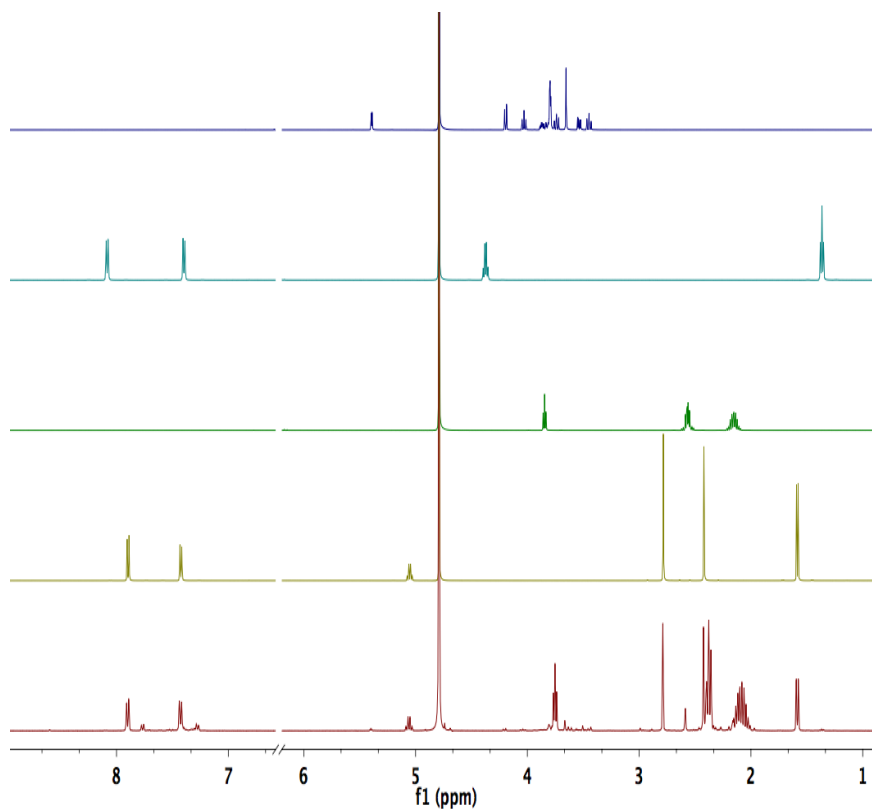


Fig. 9. ^1H NMR stacked spectrum of (a) AQ14-1 (50 mg/mL, D_2O) with the comparison conformation with authentic (b) mephedrone, (c) MSG (d) benzocaine, and (e) sucrose

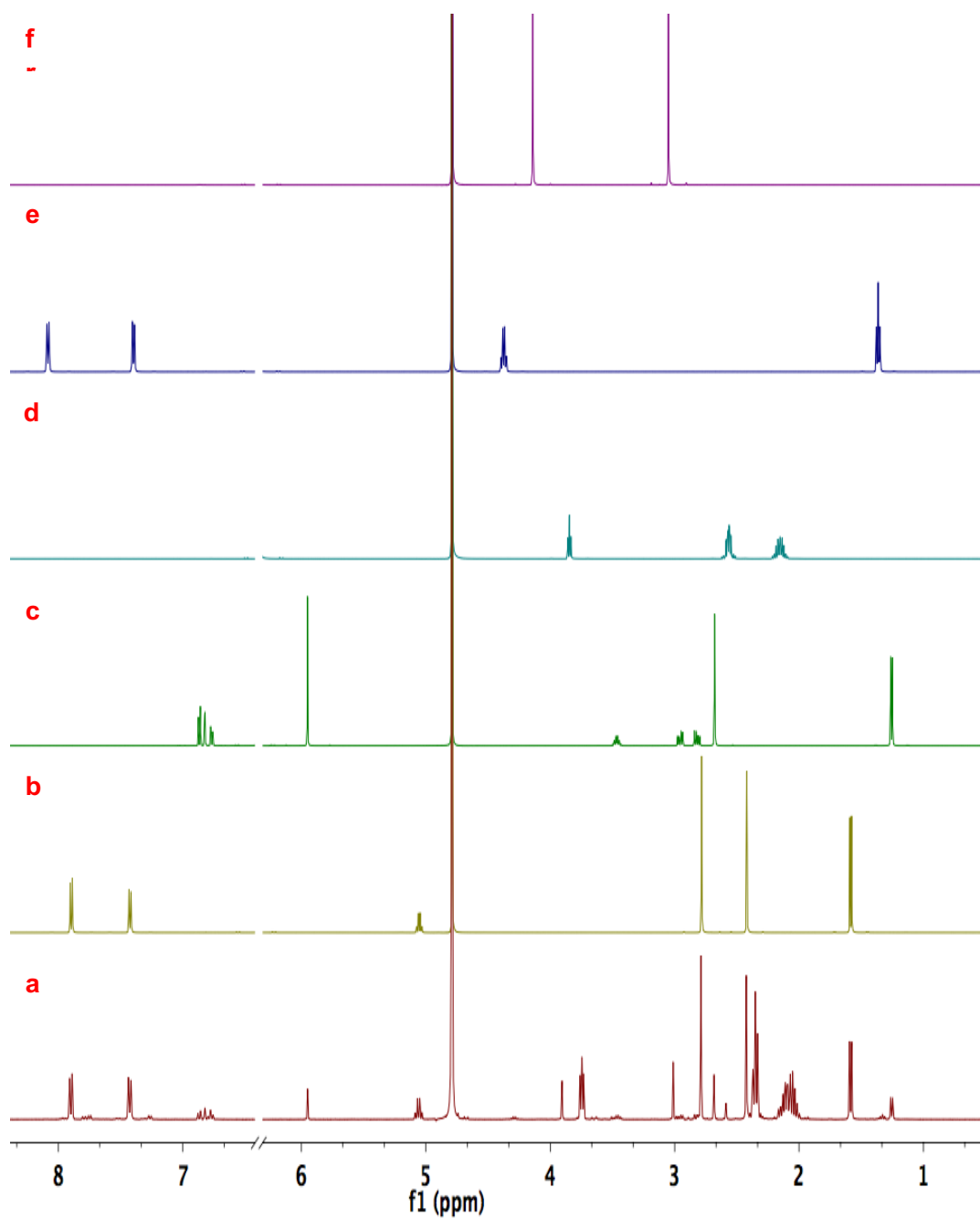


Fig. 10. Stacked ^1H NMR of (a) mephedrone sample (AI6-1, 50 mg/mL, D_2O), and compared with authentic (b) mephedrone, (c) MDMA, (d) MSG, (e) benzocaine, and (f) creatine to confirm the identity of the impurities

The ^1H NMR spectral signature of the mephedrone samples were compared to determine their similarities and differences in terms of impurities and adulterants. The stacked ^1H NMR spectra of mephedrone samples (Fig. 11) allows good comparison between the batches. Samples with MSG, creatine, sucrose and MDMA can be picked out easily. This stacked spectrum will be used at the end of this chapter to apply the chemometric analysis.

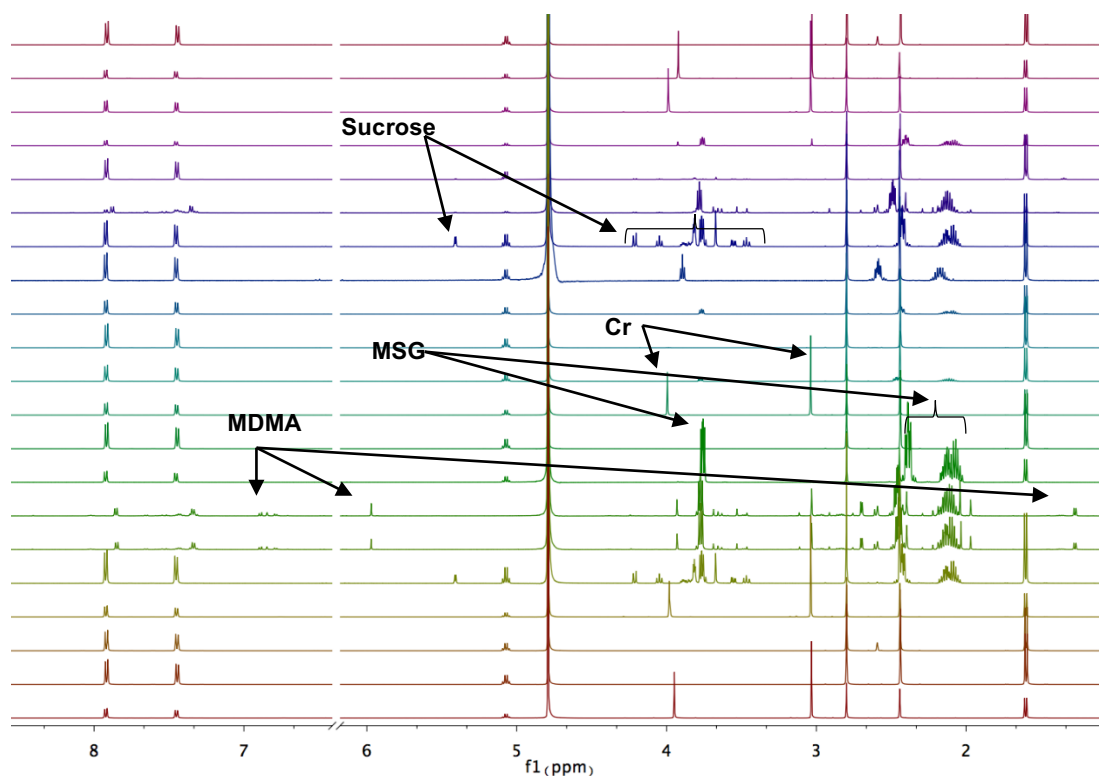


Fig. 11. Stacked ^1H NMR spectra of mephedrone samples grouped based on their impurities (D_2O peaks excluded)

5.3.1.2. 2D DOSY NMR for impurity separation in mixture of mephedrone

Illicit drugs can be impure with adulterants, by-products and solvents and this can make drug identification difficult and might lead to mislabelling. The NMR diffusion experiment (DOSY) is claimed to have the ability to separate chemical species in a solution. This technique has been used in mixture analysis for supermolecular samples [39, 40] and in food chemistry [41]. DOSY NMR was first described by Stejskal and Tanner [42] in 1965. The early use of the DOSY NMR

was to measure the diffusion coefficients in solution, and was not employed in mixture analysis until early 1990s and this follows the development of NMR spectrometers. Recently, this technique has been used to characterize heroin in seized materials [43].

Basically, molecules or ions have what so called self-diffusion, which is random translational motion, and this is based on their thermal energy. The diffusion behaviour is related to the physical properties of the target molecule (molecular weight, size, charge, etc.) and other factors related to the surroundings, e.g. temperature, concentration, and solvent. Based on this concept, compounds in mixtures can be pseudo-separated based on their individual diffusion coefficients which are inversely proportional to their molecular weight and can be measured in m^2/s [44]. This means, molecules of different MW and similar chemical shift, will diffuse at different rates and can be separated. It is also described as “NMR chromatography” but without sample preparation or method optimization [38].

DOSY NMR is measured as a collection of NMR spectroscopic experiments where the translational diffusion coefficient is measured in gradient mode using a specific pulse sequence called pulsed field gradient stimulated echo (PFGSE). In this technique, a series of pulse strength is gradually increased and a series of PFGSE NMR spectra are recorded. The resulting spectra appears as series signals decaying based on the compounds diffusion (D , m^2/s) and this can be converted into 2D-DOSY. The most common nucleus used for DOSY NMR measurement is ^1H and ^{13}C has been used as well. The horizontal dimension is a chemical shift and the vertical one for the diffusion coefficients of molecules in solutions (D , m^2/s) [45].

For DOSY analysis of mephedrone samples, the total run for each experiment was 13.28 min. The experiments were recorded under the following parameters: spectrometer frequency 500.13 MHz, acquisition time 6.8682 Sec, number of scans 16, pulse width 10.0 and relaxation delay 4.0 s. Two impure samples of mephedrone, AI6-1 and AQ14-1, were tested to check the applicability of this technique to separate mephedrone from the mixture and identify their impurities. Before applying the DOSY analysis, the impurities were identified by ^1H NMR as previously described, see Fig. 12 and Fig. 13 for AI6-1 and AQ14-1 respectively. In practice, the ^1H DOSY NMR spectrum displayed as a series of spectrum decay over the time. Fig. 14 and Fig. 15 b show the results of a series of ^1H -NMR diffusion experiments for AI6-1 and AQ14-1 respectively.

The ^1H gradient spectrum of the DOSY NMR can be transformed and aligned themselves into 2D DOSY spectrum, (Fig. 14 and Fig. 15 b). The horizontal axis represents the chemical shift, while the vertical for a diffusion coefficients values (D , m^2/s). However, in the case of mephedrone samples (Fig. 14 b), two signals are detected on the left axes. The first one is overlapped peaks for the mephedrone and the adulterants while the second one for the water (D_2O). The DOSY experiment is also a quantitative method by integrating the diffusion traces.

The DOSY experiment, samples were prepared in D_2O and two different gradient pulses tested, 1000 and 1800. Signals which belong to the same molecule will diffuse at the same rate. Solvents, e.g. water and acetone will diffuse more rapidly than any other molecule in the mixture followed by heavier molecules. The impurities, benzocaine, MSG, creatine and MDMA in AI6-1 sample can be separated and characterised based on previous knowledge from impurity profiling and their traces intensity and ppm values (Fig. 14 a). Despite the complexity in the 2D DOSY spectrum AQ14-1, the cutting agents, creatine, MSG, benzocaine, and sucrose can be identified. Unfortunately, the quantitative analysis cannot be applied in the case of AI6-1 and AQ14-1 samples due to the signals overlapping. The overlapping in these DOSY spectrums is owing to the similarity in the molecular weight/size of the mixture in the samples.

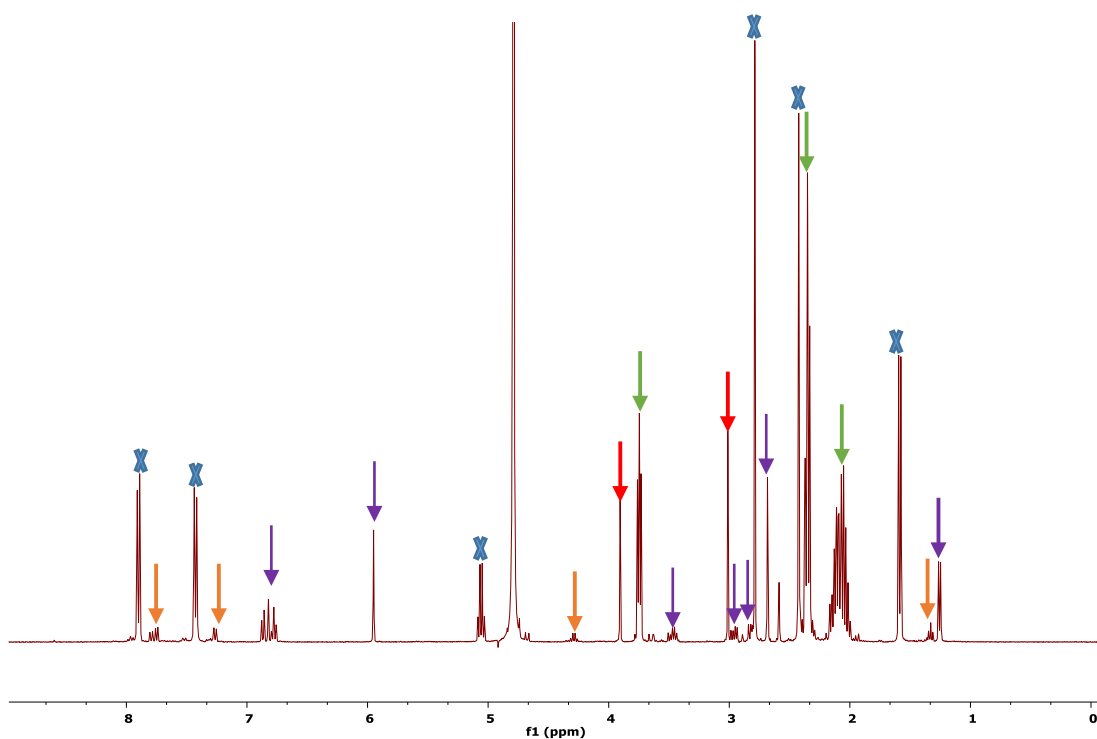


Fig. 12. ^1H NMR spectrum of **mephedrone** sample (AI6-1) contained impurities in different ratios: **MSG**, **benzocaine**, **creatine**, and **MDMA**

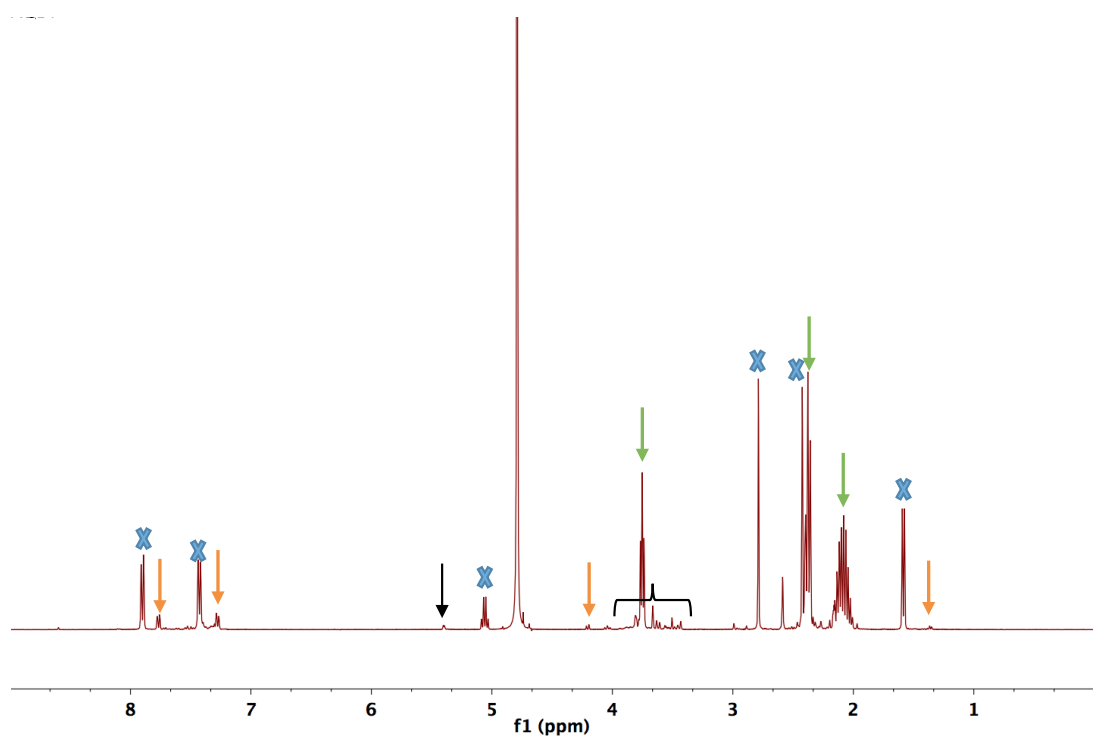


Fig. 13. ^1H NMR spectrum of **mephedrone** AQ14-1 sample in D_2O contained: **MSG**, **benzocaine**, and **sucrose**

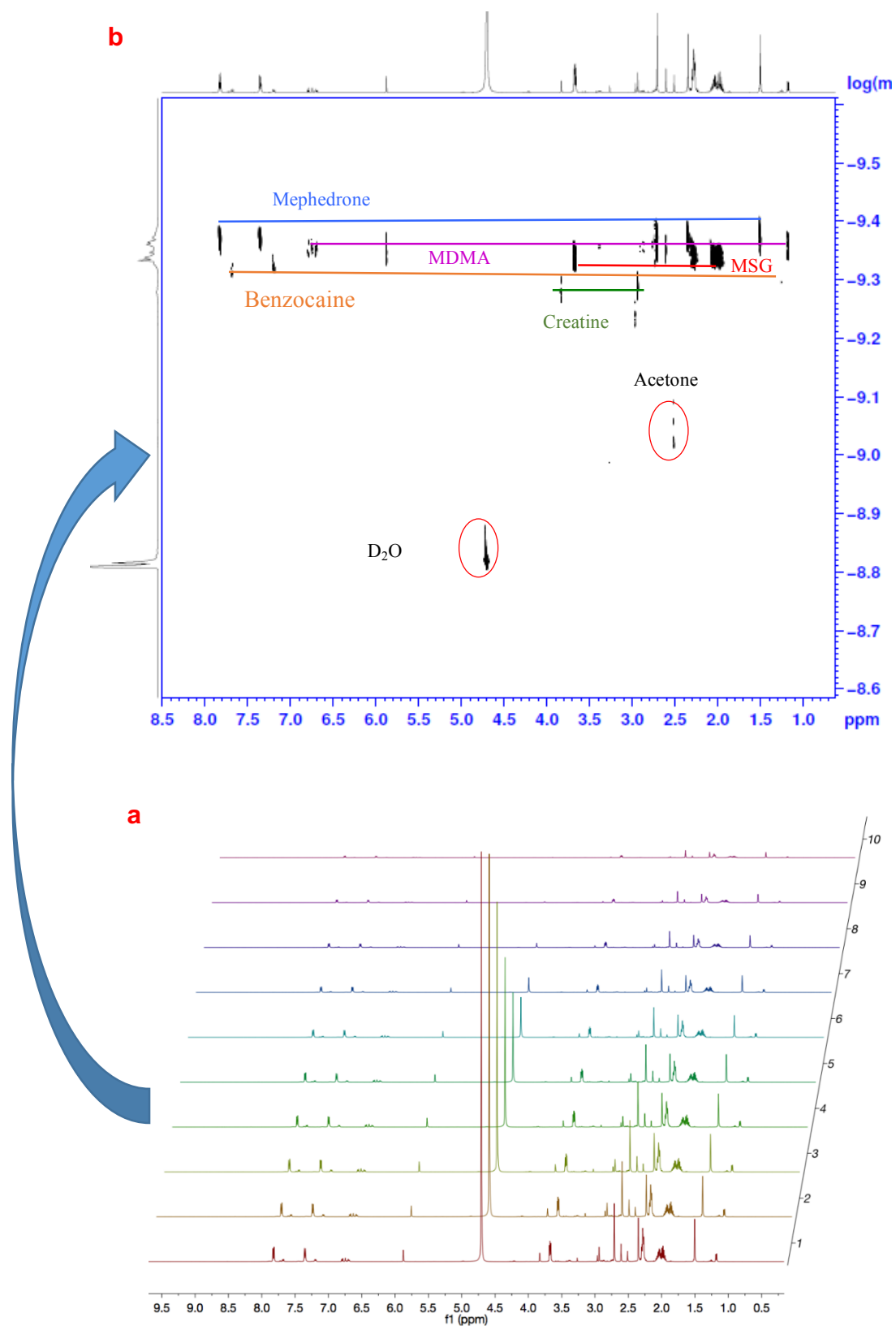


Fig. 14. (a) 1D PFG-STE experiment of mephedrone sample AI6-1 and (b) the 2D DOSY spectra in D₂O with their mixture identification

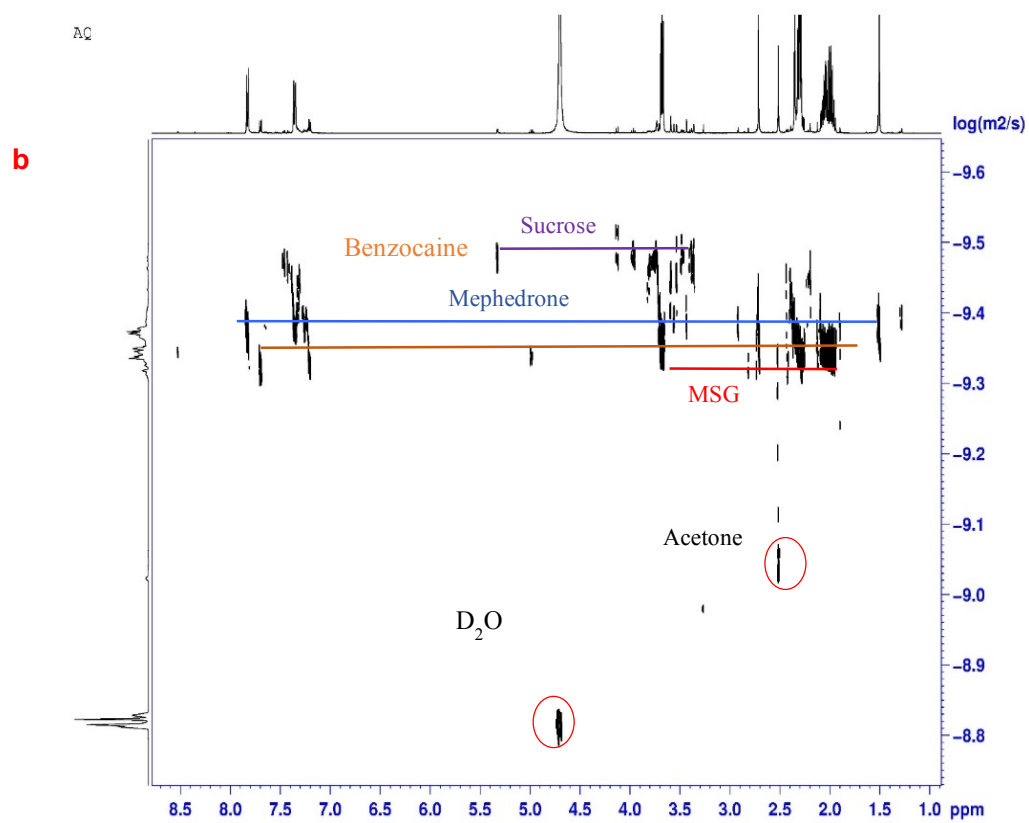
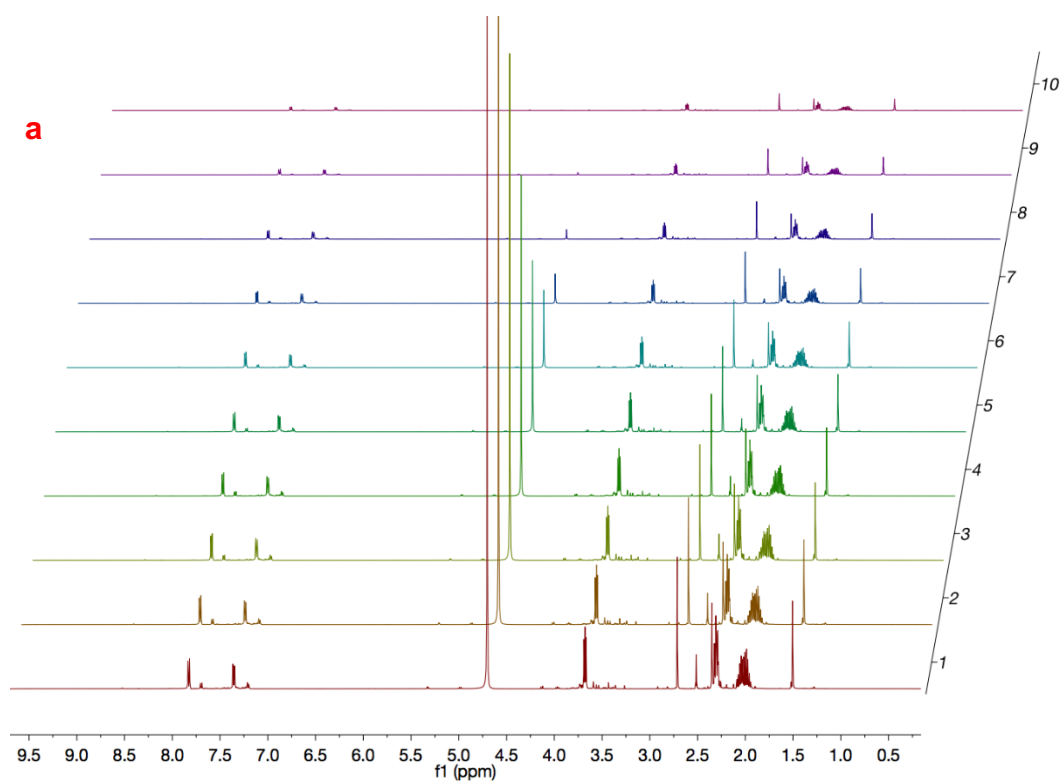


Fig. 15. (a) 1D PFG-STE experiment of mephedrone sample AQ14-1 and (b) the 2D DOSY spectra in D₂O with their mixture identification

Essentially, the ideal sample mixture for DOSY experiment is to have components with different molecular weights present in similar concentrations. In such a case the rate of diffusion of each impurity is inversely proportional to their molecular weight only, heavier molecules will diffuse more slowly. Unfortunately, this is not the case in real practise, street samples are mostly impure with different impurities and adulterants and in different concentrations. Nevertheless, the DOSY experiments were tested on two impure samples of mephedrone with 4 impurities in different ratios and in both cases the cutting agents could be identified. Furthermore, based on the impurity profiling of mephedrone samples, the street samples are commonly cut with one or two cutting agents. Samples with one and two adulterants with 50:50 ratios to the mephedrone could be ideal for this kind of separation.

Another two samples of mephedrone (Meph-1 and AK6) cut with creatine and MSG were tested by DOSY. Sample Meph-1 was cut with 1.5:2 creatine to mephedrone. Fig. 16 shows that the impurity (creatine) can be distinguished easily from the mephedrone traces in the 2D DOSY. However, only two peaks are showing on the vertical axes, one for the mixture of mephedrone and creatine and the other one for D₂O signal. Mephedrone in the second sample, AK6, was diluted with MSG, with 1.3:1 to MSG mephedrone. In the AK6 2D DOSY spectra (Fig. 17), the mephedrone traces are appeared first followed with the three peaks signals of MSG and finally the D₂O signal. In both examples the adulterants were presented at 1.5 and 1.3 ratios and were successfully distinguished from the mephedrone signals. DOSY was first introduced with optimist application to separate impurity in a mixture and this found to be useful to separate adulterants in street samples of mephedrone. This technique is the first time to be employed on separation and identification of adulterants in illicit street samples of mephedrone.

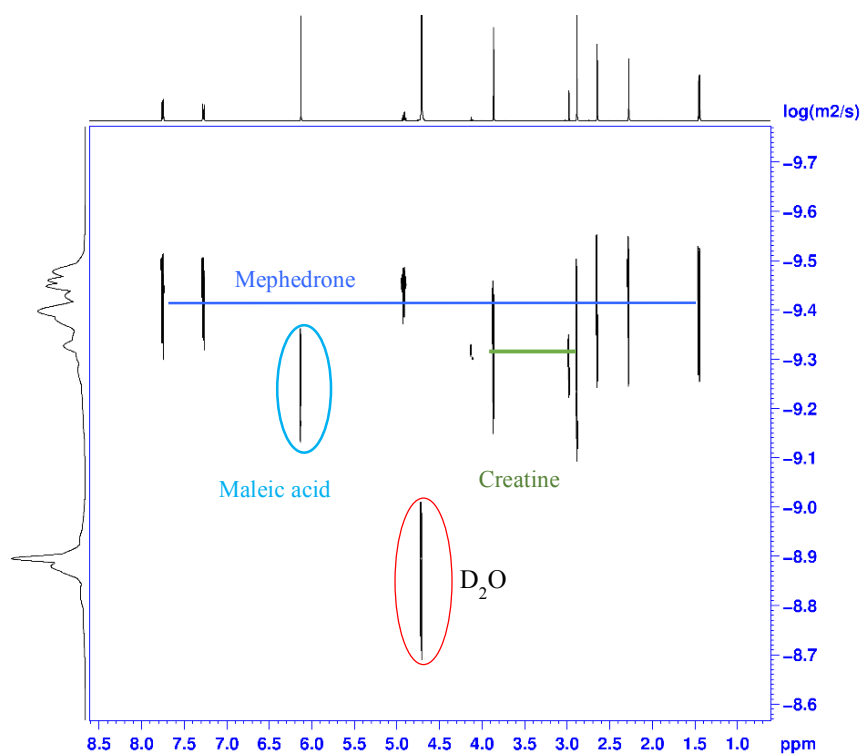


Fig. 16. 2D DOSY NMR of mephedrone sample (Meph-2, D₂O) cut with creatine

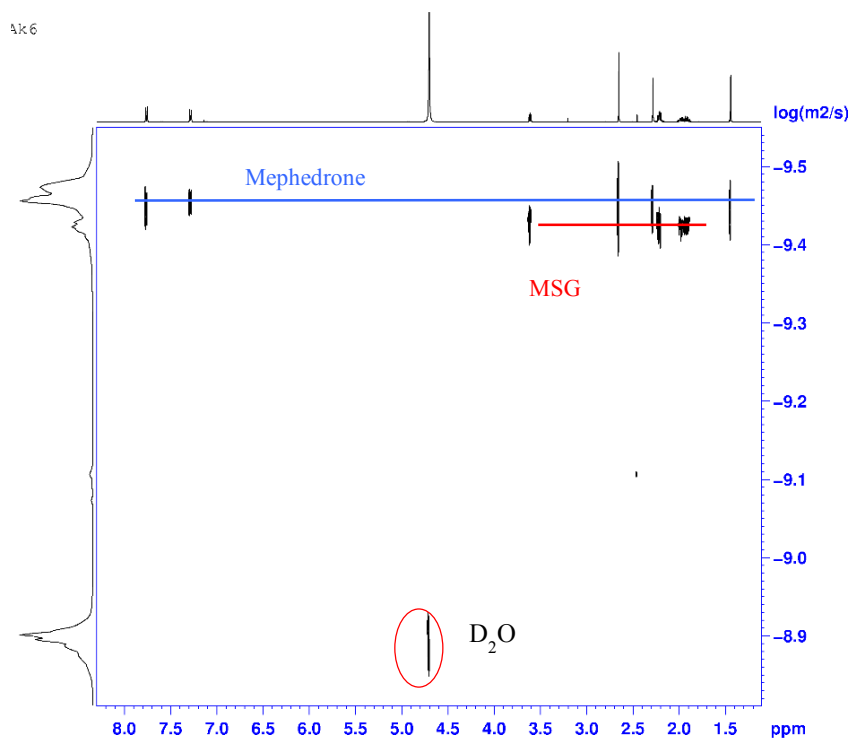


Fig. 17. 2D DOSY spectra of mephedrone sample (AK6, D₂O) cut with MSG, assignments of trace amounts

5.3.1.3. *Quantitative NMR (qNMR) analysis of mephedrone samples and their adulterants*

The major advantage of quantitative NMR (qNMR) over other spectroscopic techniques is that the integral intensity of the analyte protons directly represent the molar ratios of the molecules (Fig. 18). This is true when special care is taken on signal processing, e.g. the target molecule signals are not overlapping with any other impurity signals. To maximise accuracy, the spectrum should have a high signal-to-noise ratio (S/N) to reduce the integration error. This can be achieved by applying a suitable number of scans or use a sufficient amount of analyte [46]. For a precise quantification, the following requirements must be taken into account, sample purity, satellite peaks or heteronuclear decoupling, sample concentration, phase correction, baseline correction. The standard values of S/N for quantification are ≥ 150 for error value $\leq 1\%$ for using reference materials [47]. The accuracy of the integration procedure depends sensitively on the signal-to-noise ratio (S/N). For a high precision (standard deviation (sdv) $< 1\%$), S/N ratios of $>250:1$ for ^1H , $>300:1$ for ^{19}F , and $>600:1$ for ^{31}P should be reached at least [48].

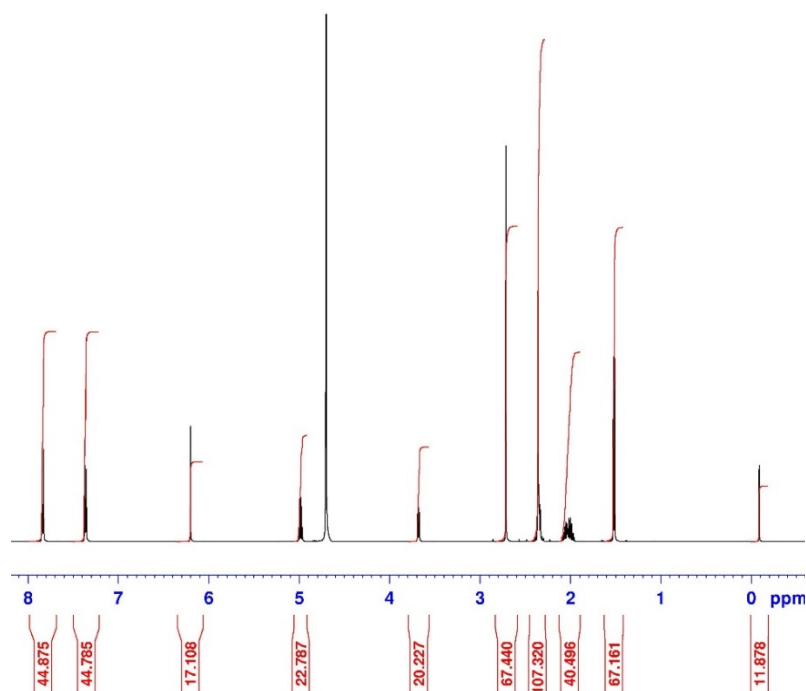


Fig. 18. qNMR of mephedrone sample (10 mg/mL in D_2O) cut with MSG in the presence of 1 mg maleic acid and 0.25% TSP and their mM values of each signal

For quantitative analysis using NMR there are two main methods, relative (ratio measurements) and absolute methods. In the ratio measurements of a mixture, the following equation is used [47]:

$$\frac{n_x}{n_y} = \frac{I_x}{I_y} \cdot \frac{N_y}{N_x} \quad (1)$$

and the fraction can be determined by:

$$\frac{n_x}{\sum_{i=1}^m n_i} = \frac{I_x/N_x}{\sum_{i=1}^m I_i/N_i} \cdot 100\%. \quad (2)$$

Where: n is the molar ratios of the two compounds x and y ,

I : the integrals

N : number of nuclei

In international pharmacopoeias, this method is used as standard method for drug mixture evaluation [48]. Furthermore, this is also used successfully for isomer quantification ratio [49].

On the other hand, for quantitative calibration approaches are used for absolute quantification, these are: Relative (Rel) 100% without calibration, Internal calibration (IC), External calibration (EC) and External calibration of the internal solvent signal (ECIC). In this study, the IC method was used for mephedrone quantification. The ECIC method was excluded as TMSP has been reported to be absorbed onto glass [43]. The IC method involves adding a known concentration of a calibrant to an accurate weight of the samples (m_x). The sample signals should be well separated from the internal standard (IS) (Fig. 18). The absolute quantitation can be calculated as following [46]:

$$C_x = \frac{I_x}{I_{Std}} \cdot \frac{N_{Std}}{N_x} \cdot \frac{M_x}{M_{Std}} \cdot \frac{m_{Std}}{m_{sample}} \cdot P_{Std} \quad (3)$$

where:

I is the integrals of the analyte (I_x) and the standard (I_{IS}), respectively.

M is the molecular weight,

P is the internal standard purity,

N is the protons number and m_s is the sample mass.

To calculate the number of moles (n_x) for each component in the qNMR spectrums the following equation was used:

$$n_x = [IS] \times \frac{I_x}{I_{IS}} \times \frac{N_{IS}}{N_x} \times V \quad (4)$$

Where:

[IS] is the concentration of the internal standard and V is the volume in 1mL.

The integral retained in the equation eq. 3 for the mephedrone quantification was the mean integral values of the six mephedrone signals protons at the following positions: 1.50 (CH₃), 2.30 (CH₃), 2.70 (CH₃), 4.90 (CH), 7.30 (CH₂), and 7.80 (CH₂) ppm. The singlet peak of the maleic acid at 6.20 ppm (2H) (methine protons) was integrated manually and quantified using eq. 3. All satellite peaks were excluded. The impurities detected within the mephedrone samples were also quantified, MSG, benzocaine, MDMA, sucrose, and creatine (see Table 1 for the ¹H NMR assignment values of mephedrone and its impurities). In cases where signals overlapped the integral value was excluded. The one-dimensional (1D) ¹H qNMR analysis was recorded using classical 1D pulse sequence (relaxation delay-pulse acquisition) parameters: Acquisition time 3.18 s, Number of Scans (NS) = 16, pulse width 10273 Hz, and 298 K data points. Basically, there is no specific rule related to the number of points is required for the quantitative analysis by qNMR. For instance, one peak is appropriate in identification of sugar in fruits while all signals are required in the case of a similar drug [50].

Integral reducibility was absorbed within a spectrum by producing similar values of the integral of each signals and IS can be calculated. To check the instrument accuracy, the internal standard purity for the maleic acid was measured. A quantitative NMR of a blank sample containing only maleic acid was tested and gives the value of 99.0% which equates to the purity provided by the manufacturer (Aldrich). The practical values from the integral obtained for the maleic acid at 6.20 ppm is 8.64 ± 0.07 mM which is equal to 1.002 mg/mL. The signal to noise (S/N) values were $\geq 30\,000$ measured for all the qNMR spectrums of mephedrone and these were far bigger than 20 000 (S/N) which is equal to the relative uncertainty percentage 0.05% [51].

The street mephedrone samples were presented in various levels of purity, ranging from 87.3% to as low as 2 % (Table 3). For instance, GA6 mephedrone samples contained 87.3% mephedrone, while K2 has just 7.8% mephedrone (Fig. 19). Purity profiling more or less reflects the impurity values of the samples e.g. if the sample has 40% impurity that means the purity for the target compound is 60%. However, the purity/impurity correlation does not always reflect the total content of samples. Undetectable materials e.g. inorganic impurities and overlapping signals can make the impurity profiling difficult. Materials such as silica gel, NaCl, and some polymers can escape the NMR investigation. Results from the qNMR results of the purity/impurity percentages and the mM ratios are shown in Table 3.

Samples with high purity, close concentration values, same impurity amount, and different sources may link to each other and to a manufacture. Samples GA6 and AM3-1 were from separate amnesty bins, Glastonbury and Bristol night club, respectively. Both samples are pure with close concentrations values, 39.8 mM and 39.3mM of mephedrone. This similarity can be used to advocate that GA6 and AM3-1 might link to the same manufacture as pure batches. The purity of the 4 pure samples of mephedrone were between 71 and 87 %, one from Glastonbury, and one from Bristol night club besides two seized samples from Bristol. Drug dealers usually received their drugs in bulk amounts and attempt to split them into small bags to be delivered, and this might explain the high similarity between them. The same explanation can be expressed on samples AM7-1 and AM7-2 from the Bristol night club amnesty bin. The qNMR confirmed that these two samples have close mephedrone purity percentage, 17.6% and 21.9 % respectively. Moreover, the same adulterants were detected and in very similar values, see Table 3. With respect to impurity quantification in AQ14-1 and AI6, both samples have many overlapping peaks and few were used for the calculation.

Regarding to the mephedrone dose, this is dependent on the method of administration, body weight and other factors. For example, the insufflation dose of mephedrone is between 20 to 80 mg and can as high as 125 mg [12]. A typical single dose of mephedrone is between 5-250 mg, and re-dosing with 0.5-1 g is normally desired due to the short effects of mephedrone. The effects are seen a few minutes after insufflation and longer after ingestion, 15-45 min and last about 2-3 h [7]. From the qNMR results in Table 3, mephedrone concentration ranged from 0.2-8.5mg/mL. The qNMR was shown to be fast, practical, non-destructive for

quantifying mephedrone and its impurities. However, very low quality mephedrone samples can be difficult to quantify due to overlapping peaks.

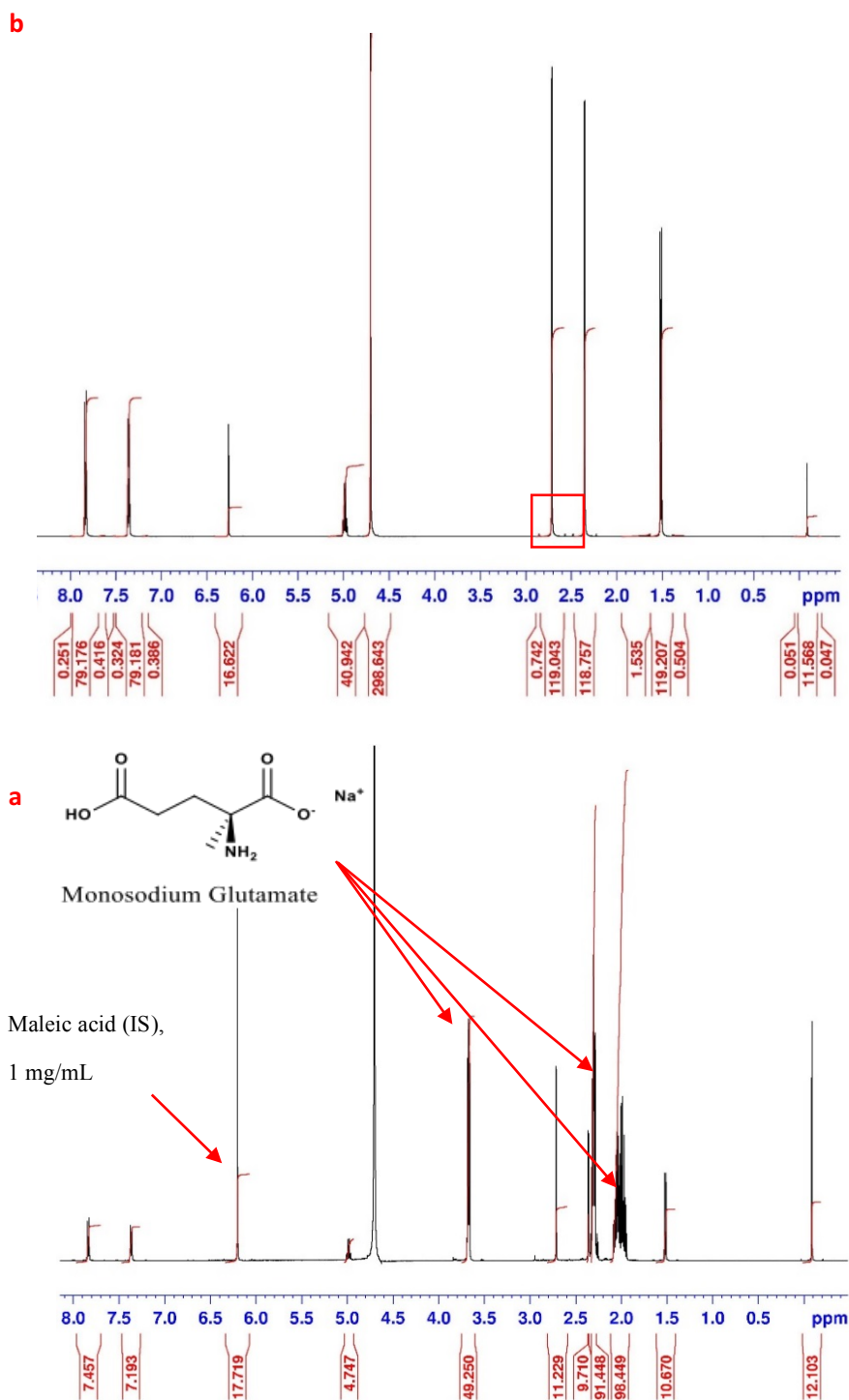


Fig. 19. ^1H NMR spectrum of (a) GA6 87.3% pure, and (b) AK2 sample contained only 7.8% mephedrone (both spectra are in D_2O)

Table 3

Mephedrone samples and their impurity quantified by 1D ^1H qNMR

	Sample cod	SD of integrals	Meph mM	Meph mg/mL	Meph purity, %	Adulterants, purity %
1	GA6	0.23	39.8	8.5	87.3	-
2	AM3-1	0.10	39.3	8.4	85.8	-
3	M.t	0.24	32.29	6.9	71.3	-
4	Se.15	0.49	37.2	7.9	79.6	-
5	AK4	0.26	36.9	7.8	80.8	MSG 0.40
6	AM7-1	0.16	8.43	1.8	17.6	MSG 40
						MDMA 0.49
						Sucrose 17.5
7	AM7-2	0.36	10.23	2.2	21.9	MSG 40.6
						MDMA 0.49
						Sucrose 14
8	AC6-1	0.39	26.1	5.6	56.6	MSG 24.4
9	AK6	0.15	22.5	4.8	48	MSG 34
10	AK2-1	0.44	3.87	0.82	7.8	MSG 80.1
						Creatine 3.7
11	GE9-1	0.46	8.41	1.8	18.2	Creatine 3.7
						MSG 58
12	AK11-1	0.35	1.66	0.35	3.7	MSG 4.4
13	M1-1	0.54	18.3	3.9	38.2	Creatine 45
14	M1-2	0.29	18.6	4	38.6	Creatine 50.4
15	M2-1	0.6	16.4	3.5	35.4	Creatine 42
16	M3-1	0.28	17.1	3.6	35.7	Creatine 51.6
17	Se.15-2	0.66	20.13	4.3	43.0	Creatine 41.8
18	AQ14	0.15	0.98	0.2	2.05	MSG 38.5
						Sucrose 14.2
						Benzocaine 0.45
19	AS4	0.6	37	7.9	80.6	Sucrose 6.7
20	AI6.1	-	15.04	3.2	33	MSG 67

						Creatine 2.8
						MDMA 2.56
						Benzocaine 0.6
21	AI6.2	-	15.61	4.8	34.26	MSG 60.6
						Creatine 3.2
						MDMA 1.37
						Benzocaine 0.57

Although the mephedrone samples look pure in the ^1H NMR, the purity was low, e.g. mephedrone sample code AK11 (Fig. 19 a) shows to be pure by ^1H NMR but the calculated purity was 87.3%. This might be due to water in the mephedrone. The average purity of the mephedrone samples from a Bristol night club amnesty bin was 39.3%, while Glastonbury amnesty bin was higher with 52.7%. Seized samples of mephedrone from two different areas in Bristol were 43.8% (2013) and 61.3% (2015).

5.3.2. Chemometric analysis of the mephedrone samples

As the levels of drugs and impurity vary between seized illicit drugs, this can be used to link between different seizures. Visual comparison between NMR spectra of seized samples can be difficult, and time consuming. Integrating both impurity profiling and data mining (chemometric) could help toward building a national and international database for illicit drug production and trafficking [52]. First chemometric model was made by Johnston and King in 1998 to predict the origin of heroin [53]. For impurity profiling of illicit drugs various statistical and chemometric techniques have been used as exploratory classification methods [52]. The most common techniques are principal component analysis (PCA) and hierarchical cluster analysis (HCA). Unlike PCA, HCA presents all variations on a data set and not only the percentage values.

Mephedrone samples were classified based on their impurity/purity by ^1H NMR. Although it is possible to visually differentiate between mephedrone samples by using ^1H NMR, minor differences might be missed. PCA and HCA analysis were applied to the qNMR data in D_2O for 21 samples of mephedrone. To prepare data

for PCA and HCA test, all the spectra had to be referenced, normalised to the sum values, solvent and IS peaks were excluded and a range adjust from 00 to 9. Each spectrum was cut into small parts, each equal to 0.04 ppm. The ^1H NMR spectra were then converted into numeric sets of data to apply the PCA and HCA analysis.

The data were analysed by using PCA to determine whether there is one main component that can account for the correlated variance among the samples. The PCA is usually tested in sequential steps to know how many components to extract in the analysis. The scree plot was performed showing a break after the first four components, i.e. four components described the majority of the samples (Fig. 20, a). The scree plot looks at variance versus number of factors. Basically, the components on the steep slope are extracted. The result shows that 71.8% of the 21 mephedrone samples are similar and arranged under a main cluster, PCA1 (Fig. 20, b). While PCA2, 3, and 4 presented at 10.4, 5.9, and 3.6% of the variance among the data, respectively. The components are allocated on the shallow slope are contribute little to the solution. Further analysis is required to test whether the component 4 might be worth extraction or not.

Pattern matrix component is can be useful to identify and understand the nature of the components (Table 4). The first eight samples are loaded quite nicely on the first component, ≥ 0.90 , that means the variations between these samples are quite low. The impurity profiling of the samples included in PCA1 was examined by ^1H NMR and all these samples were $\geq 48\%$ pure or contained MSG as cutting agent (Fig. 21 (a and b)). Samples AK11, AM7.1, and AM7.2 are less pure, than the other samples load on PCA1. The PCA analysis has successfully grouped the samples based on their cutting agents. All mephedrone samples AI6.1, AI6.2 and AQ14 are loaded better on the second component PCA2. The first two samples in PCA2 were cut with a combination of MSG, creatine, MDMA, and benzocaine, while AQ14 was cut with MSG, sucrose, and benzocaine. Component 3 has 5 major positive loading for samples cut with creatine. While samples cut with creatine and MSG are loaded on PCA4. Both GE9 and AK2 are loaded negatively on the first three components, but allocated strongly on the component 4 and the ^1H NMR shows the samples were cut with both MSG and creatine (Fig. 22).

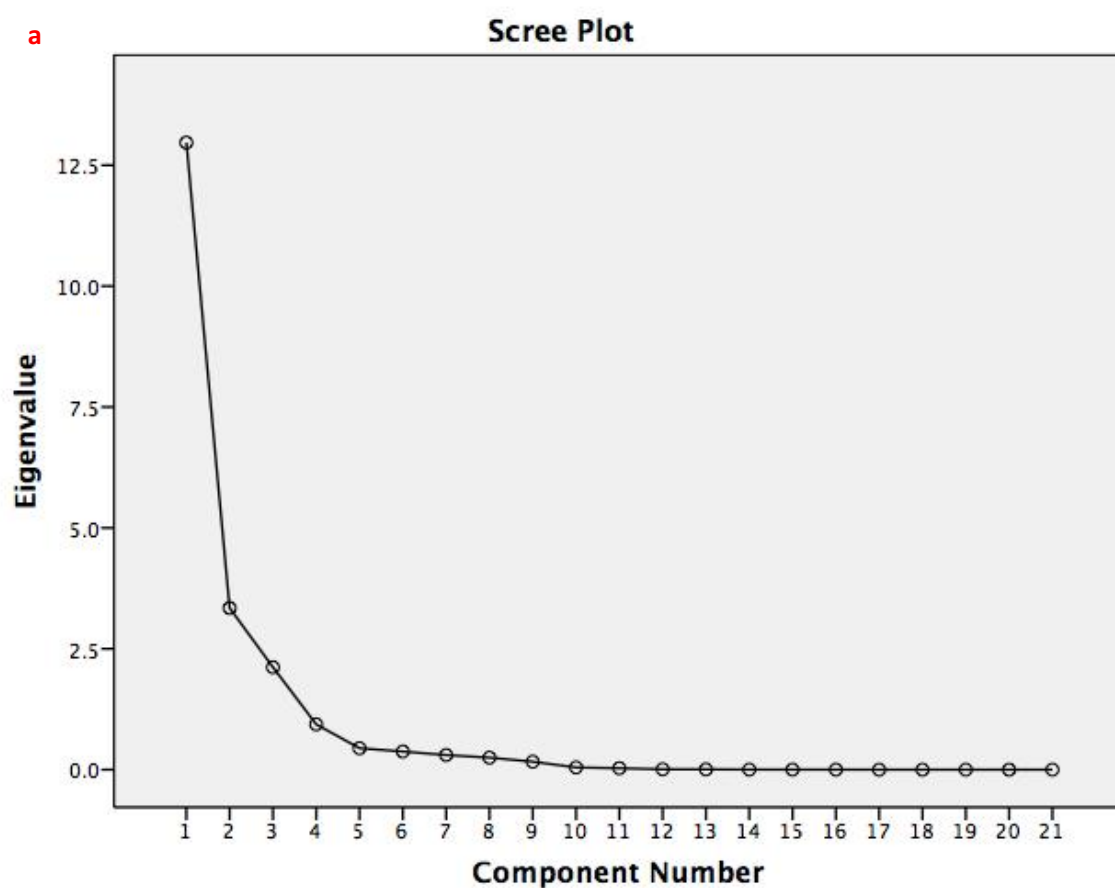
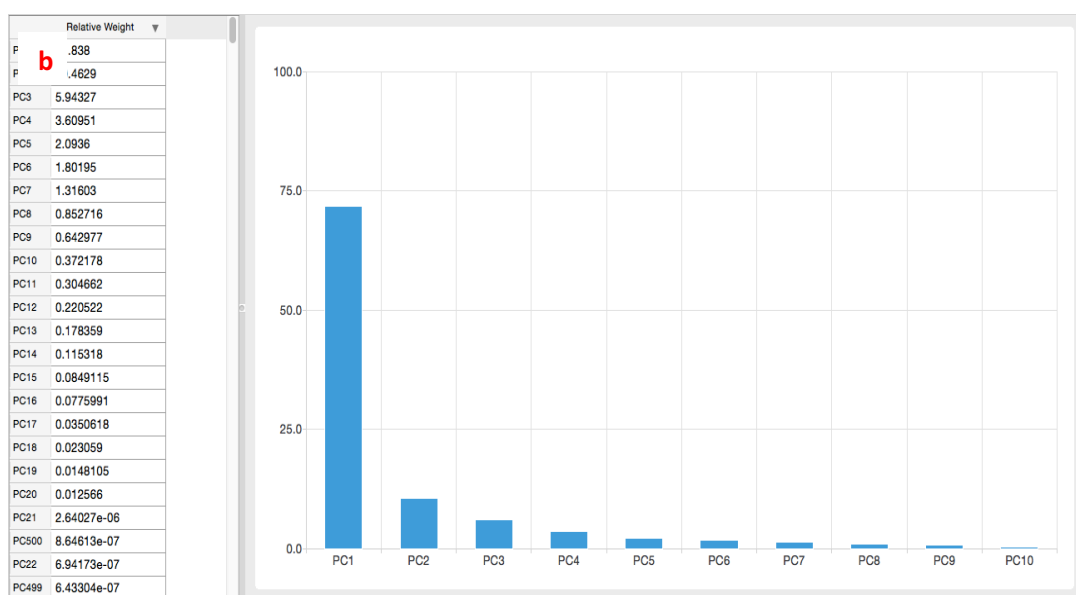


Fig. 20. (a) Scree plot of the dataset of mephedrone showing the main component numbers (four) and (b) the samples percentages for each component

Table 4. Pattern matrix extraction method using principal component analysis

	Component				
	1	2	3	4	5
M.t	.986	-.075	.044	-.001	.048
AM3	.984	-.097	.047	.005	.002
GA6	.984	-.096	.048	.003	.001
Se.15	.983	-.089	.027	-.002	.052
AS4	.982	-.077	.052	-.005	-.019
AK4	.981	-.090	.054	.002	.001
AC6	.912	.141	.057	-.011	-.012
AK6	.903	.110	.034	.058	-.110
AK11	.780	.163	.031	.007	.386
AM7.2	.737	.316	-.012	.111	-.255
AM7.1	.679	.349	-.026	.115	-.298
AI6.1	-.103	.964	.072	.007	-.070
AI6.2	.001	.904	.048	.082	-.148
AQ14	.043	.879	-.053	.033	.217
M1.3	-.090	-.015	1.027	.000	-.092
M1.1	.027	-.025	.966	-.001	-.091
M3.1	-.034	.076	.892	.014	.207
Se.15.2	.158	-.042	.886	.003	-.086
M2	.088	.026	.841	.003	.063
AK2	-.122	-.036	-.024	1.052	.042
GE9	.336	.081	.096	.746	-.037

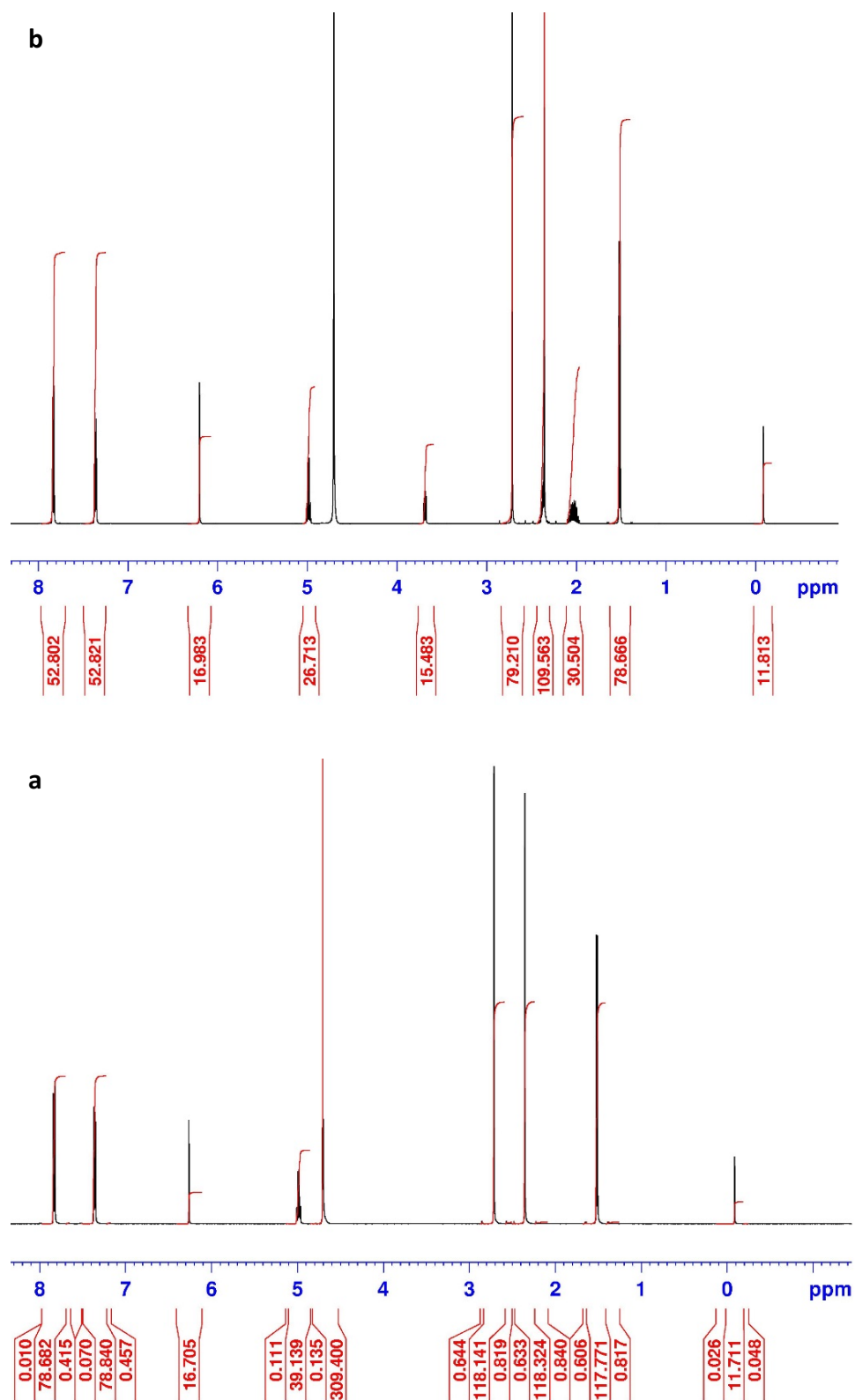


Fig. 21. Mephedrone samples in the first component (PCA1), (a) pure sample of mephedrone (AM3-1, 87.3%), (b) mephedrone cut with MSG (AC6, 57%)

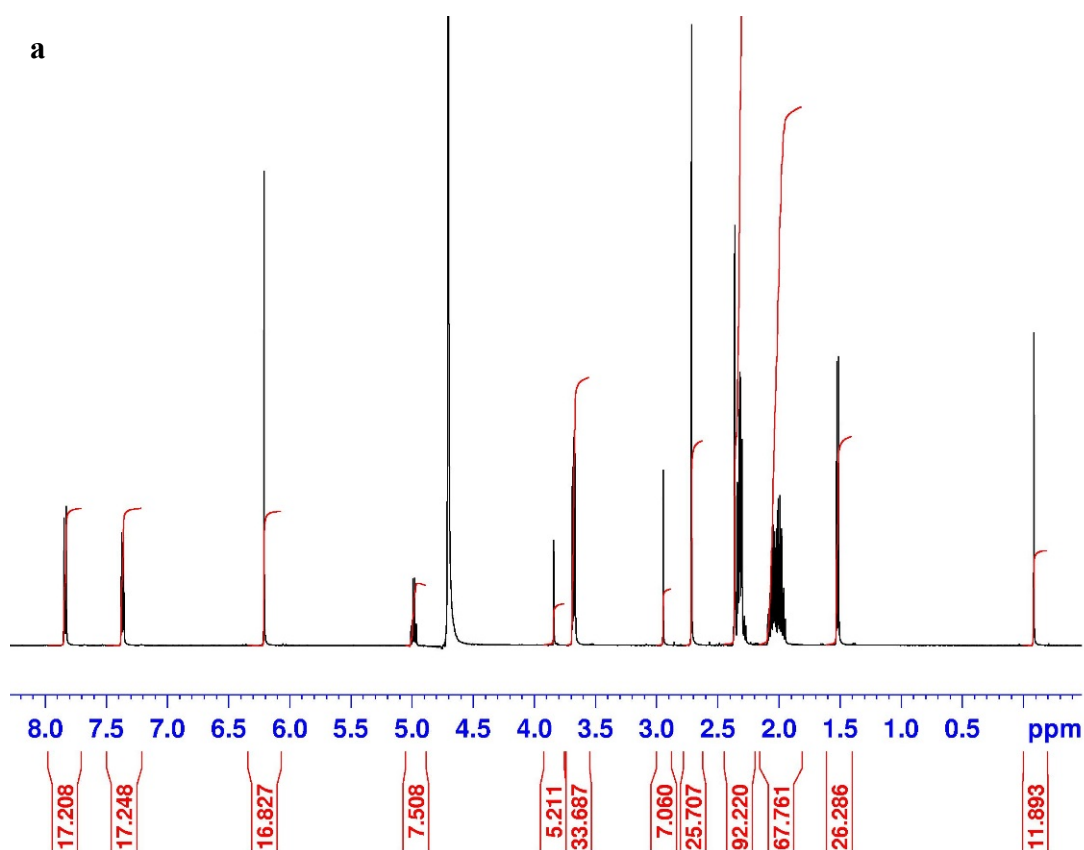
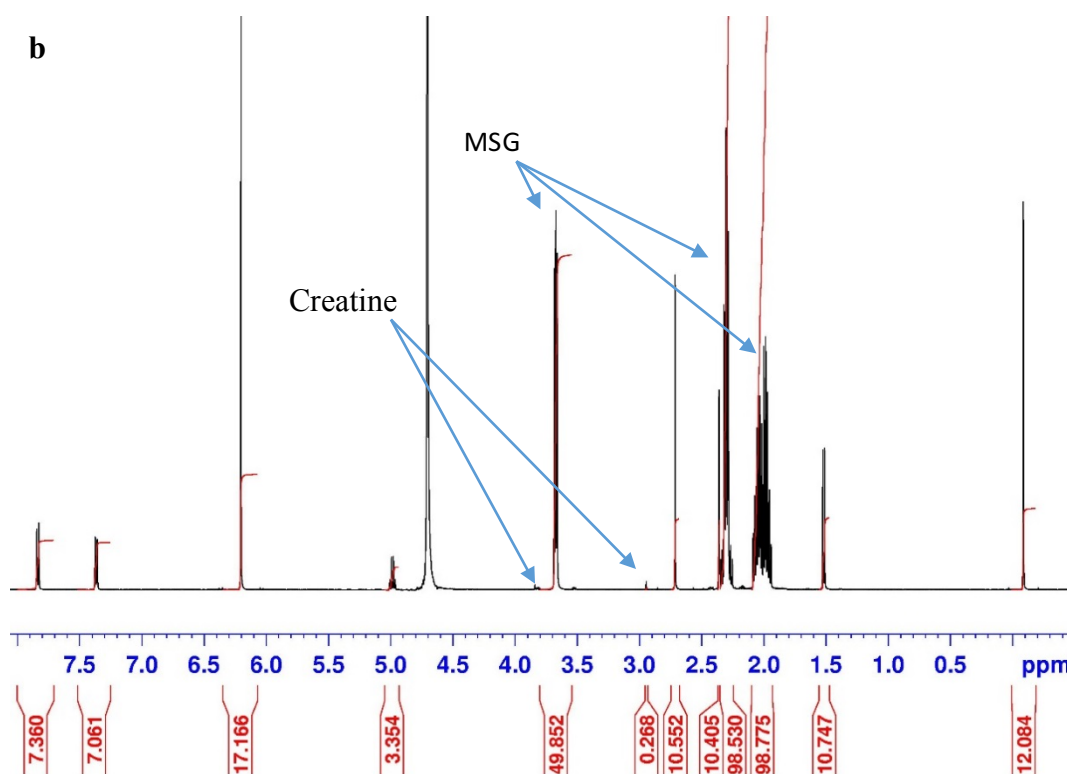


Fig. 22. (a) GE9 and (b) AK2 samples loaded on PCA4 were both cut with MSG and creatine

To identify possible linear correlations between different PCA, scatterplot matrix is a great starting point. The plot is showing the correlation between the variables (PC scores) on the vertical axes with the horizontal one. There is a positive correlation between PCA2 and PCA3 showing gradual distribution from top right to the bottom left. Other possible negative correlation is showing between PCA3 and PCA1. There are no clear correlations between PCA5 and the other PCA in the plot matrix. Based on the information extracted from the scatterplot matrix, the data can be presented better by using a 3D correlations PCA1, PCA2 and PCA3.

In the 3D PCA scores plot, all pure samples were grouped together with samples cut with MSG only. GE9 and AK2 scatter differently with GE9 allocated closer to the pure PCA1 (pure samples and samples cut with MSG), while AK2 scatter closer to PCA3 (less pure mephedrone sample). NMR specters of GE9 (Fig. 22, a) and AK2 (Fig. 22, b) show that both are containing same cutting agents but in different mephedrone concentration. One explanation of this that GE9 has more mephedrone (18.2%) while AK2 contain only (7.8%). The rest of the samples distribution shows good agreement with the pattern matrix component (Table 4).

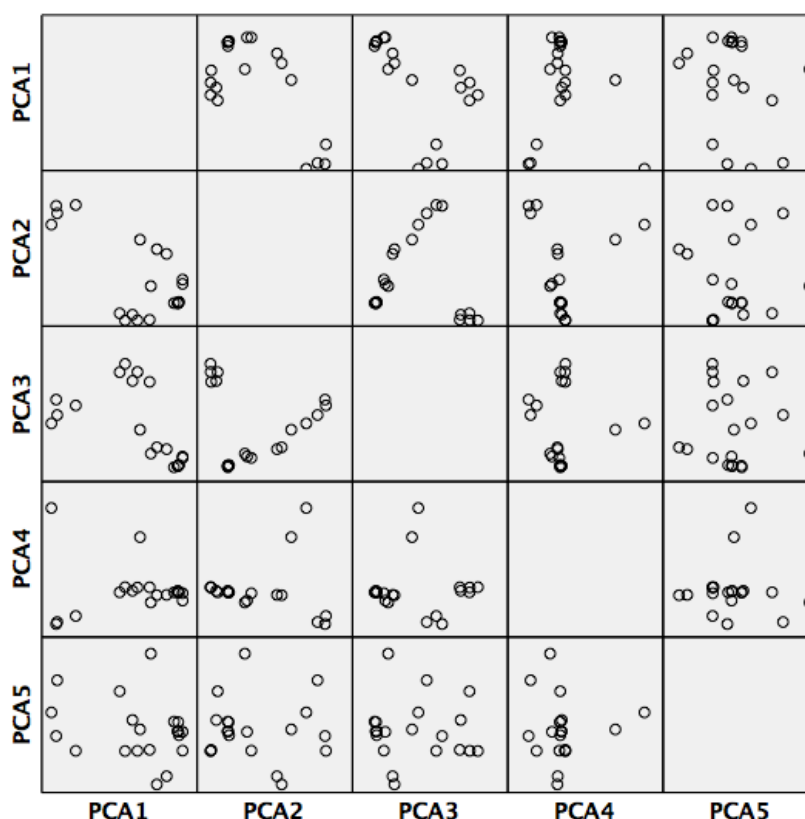


Fig. 23. Data set trees scatterplot matrix of the first five PCA

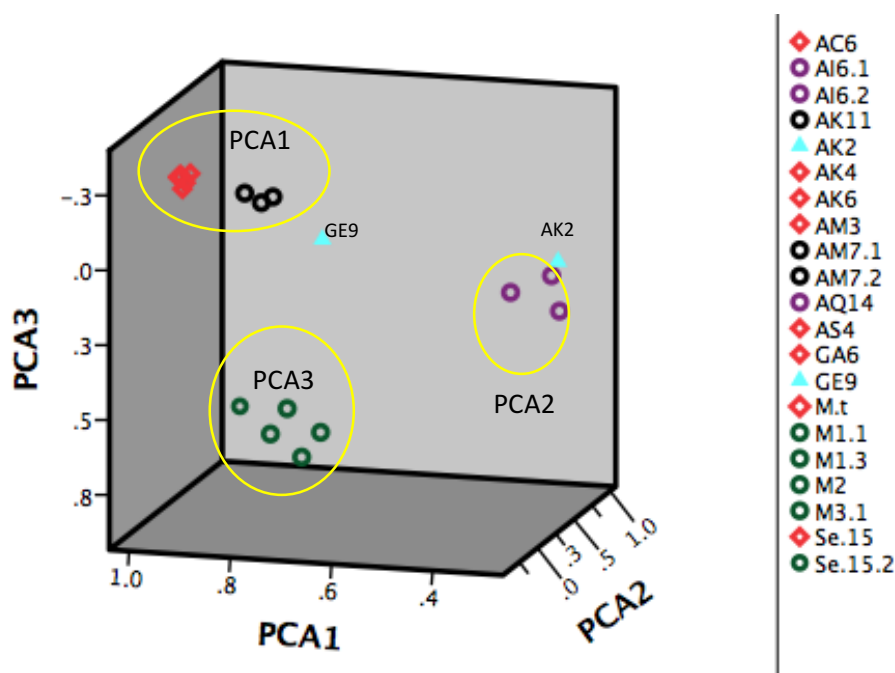


Fig. 24. PCA scores plot of PA1, PCA2, and PCA3

More discrimination can be achieved between the mephedrone samples by applying the HCA analysis, dendrogram. The vertical axis represents the sample names and clusters. The dissimilarity between clusters are defined based on the distance on the horizontal axis. The first noticeable clustering in the dendrogram results (Fig. 25) that all the pure mephedrone samples ($\geq 80\%$ purity), are branched in one separate cluster. While the other impure samples are cluster into subgroups based on their similarity and differences.

The dendrogram tree can be cut at two different places, 15 and 5 with two and four clusters, respectively. The cut at 15 will divide the data into two main clusters, one including the mephedrone samples with $\geq 80\%$ purity and the other representing the less pure mephedrone samples. Samples in the second cluster (using the cut at 5) are divided into two sub-clusters representing samples cut with creatine only at different percentages (Se.15-2, M1.3, M1.1, M2, and M3.1). The third cluster contain two sub-groups (two samples each) are fused at higher distances. Both of these groups are sharing MSG as cutting agent, but AM7.1 and AM7.2 have less mephedrone concentration besides sucrose and traces of MDMA. The last cluster contain 6 sets of samples are branched into two groups. All these samples in this cluster contain mephedrone samples with purity percentage ranging from 34.2%

to 2.0%. The second group in the last cluster AI6.1 and AI6.2 were cut with MSG (67 and 60.6%) creatine (2.8 and 3.2%) MDMA (2.56 and 1.37%) Benzocaine (0.6 and 0.57%), AQ14 was cut with MSG (38.5%), Sucrose (14.2%), Benzocaine (%), and AK11 cut with MSG (4.4%). Visual investigation of AK11 samples (Fig. 26) gives no clear explanation for clustering this with AI6.1, AI6.2, and AQ14. The possible answer is that AK11, AQ14, AI6.1, and AI6.2 are presenting at low mephedrone levels or other the similarity could be due to minor compounds were presented in the samples. The dendrogram was successfully distinguished between the pure and impure mephedrone samples.

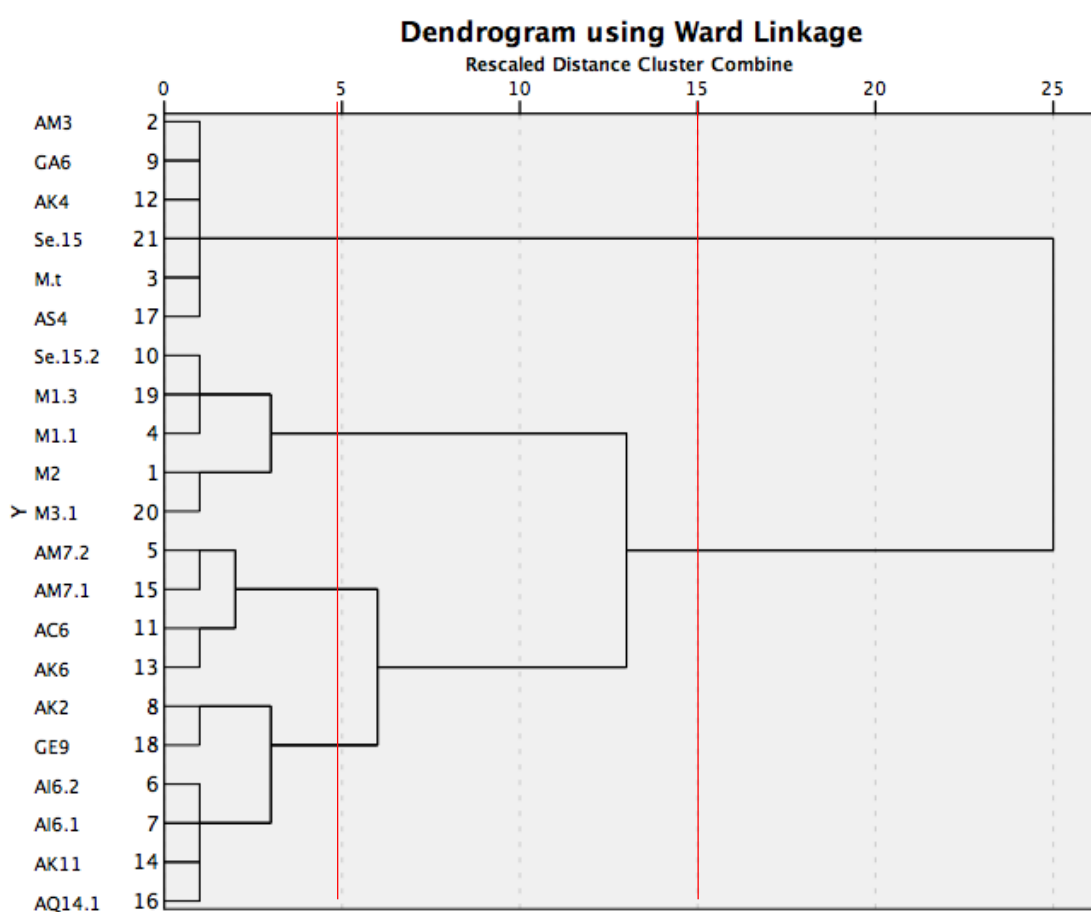


Fig. 25. Dendrogram using average linkage between samples

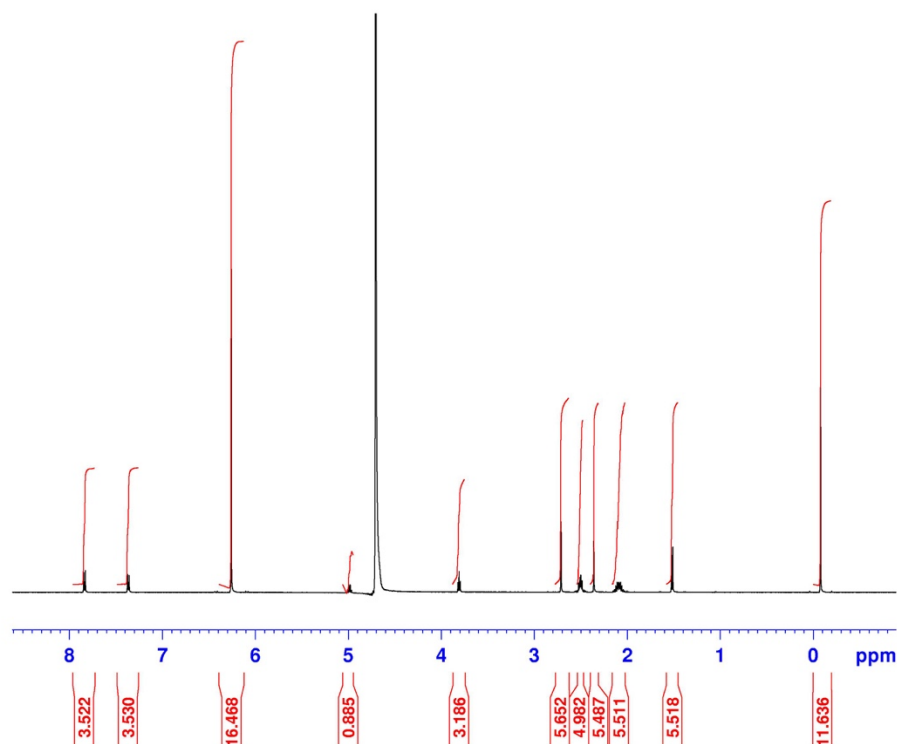


Fig. 26. qNMR of AK11 contain 3.7% mephedrone and cut with 4.4% MSG

5.4. Conclusions

Unique and a powerful NMR analytical techniques were introduced for impurity profiling of street mephedrone sample using ¹H NMR, 2D DOSY and quantitative NMR (qNMR). Both mephedrone and their adulterants were successfully identified and quantified without calibration, reference materials, sample preparation and this cannot be offered by any chromatography. Molecules not detected by NMR are limited compared to other chromatography and spectroscopy methods. The qNMR performed illustrates the capabilities of this technique for both quantitative analysis (purity/impurity quantification) and structure characterisation. The qNMR analysis of the mephedrone samples could be further improved by decoupling the sidebands (¹³C satellites peaks) to simplify the spectrum and facilitate assignments. Going forward, it will be important to quantify these mephedrone samples using chromatographic techniques. Hence, method development in these other technique to be able to detect mephedrone and adulterants will be required. On the other hand,

2D DOSY NMR proved to be a promising method in terms of impurity separation with limitations linked to the overlapping peaks. In reality, most of the mephedrone samples were cut with only one or two adulterants. Consequently, this increases the chances of separating the impurities in the mephedrone when using DOSY.

Mephedrone samples were received in powdered and crystal forms as samples seized by the police and as a part of the analysis of amnesty bins (Bristol night club and Glastonbury music festival). Our impurity profiling data of mephedrone suggest that the drug dealers cut the mephedrone with safe, available and cheap materials e.g. creatine, MSG and sucrose. However, pharmaceutical agents e.g. benzocaine, a topical anesthetic, is also used, perhaps to mimic the numbing effect of cocaine on the tongue/lips so that it might be sold as cocaine. Moreover, benzocaine is reported to be used to cut other cathinones, e.g. flephedrone and this might link the mephedrone to other drugs distribution and production. The most common administration method of mephedrone is insufflation with high and multiple doses and harm associated with adulterants such as benzocaine and MSG and creatine must be taken into account. Other recreational drugs e.g. MDMA are also used as adulterants for mephedrone in small quantities.

Simultaneous determination and quantification of street mephedrone samples and their adulterants by NMR was cross-validated with statistical visualization examinations. The principal component analysis (PCA), scree plot, and dendrogram effectively classified mephedrone samples with good agreement with the ^1H NMR impurity profiling analysis. This kind of analysis of mephedrone street samples (impurity profiling, quantification and statistical analysis) can provide valuable information about availability and purity with possible link between samples and maybe to the source of supply to aid law enforcement officers.

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Chapter 6

Impurity profiling of street ketamine samples

6.1. Introduction

Ketamine is a unique anaesthetic and analgesic due to possessing a wide margin of safety in its use and the ease of its administration [1]. The WHO rate ketamine highly, listing it in the Model List of essential anaesthesia medicines for surgery and required in Emergency Departments for use in humans and animals [2, 3]. Ketamine has various medical uses ranging from anaesthesia [2], treatment of heroin and alcohol addiction [4], to more recent promising applications in the treatment of resistant depression and severe asthma [5, 6, 7, 8]. Ketamine was introduced in 1965 as a veterinary and human anaesthetic [9]. This was made as an alternative to phencyclidine (PCP, “angel dust”), in the hope of providing a new anaesthetic with shorter psychosis and less delirium. The advantages of ketamine over phencyclidine (Fig. 1) are that ketamine can produce deep analgesia and amnesia without any slowing of the heart rate or adverse effect on breathing [10].

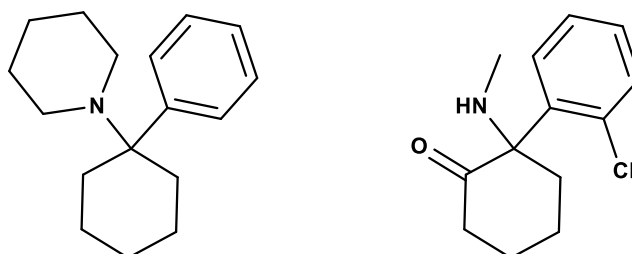


Fig. 1. Phencyclidine (left) and its derivative ketamine (right)

Following use over the last 51 years, ketamine is still a drug of choice in pain management [11]. However, this drug is abused for its hallucinogenic effects [12]. It can also produce euphoric effects similar to cocaine and cannabis [13]. The non-medical use of ketamine was reported in the 1960s [14], but it was not common in Europe until the 1990s when it appeared as an adulterant to ecstasy tablets [15]. Ketamine has become a common recreational drug, especially at rave parties [16, 17]. Street names of ketamine include: Special K, Kit Kat, Cat Valium, etc. Among ketamine abusers, the youngest are the majority [12] who suffer from irreversible

damage to the bladder, urinary tract, kidneys [18], and addiction [19]. It has also been reported that ketamine combined with other drugs, including alcohol, can lead to death [10]. Ketamine is still not internationally controlled and this is to ensure the accessibility of ketamine for medical uses [20].

According to the British National Formulary (BNF), the currently authorized hospital formulation of ketamine in the UK is KetalarTM (ketamine hydrochloride, provided by Pfizer Ltd, Kent, UK) [21]. While veterinary ketamine is available in nine trade names: Ketavet[®], Narketan[®], Anaestamine[®], Anesketin, Nimatek, Ketaset, Ketamidol[®], Ketastesic, and VetalarTMV [22]. Commonly, in the UK, ketamine is a racemic mixture, composed of equal amounts of levorotatory enantiomer, *R*-(-)-ketamine and dextrorotatory, *S*-(+)-ketamine. In controlling the pain of postoperative patients with ketamine, drug conformation (i.e. 3D shape) and, in this case, absolute configuration (i.e. optical isomers) play a major role, with a four-times more potent *S*-enantiomer compared with the *R*-(-)-ketamine enantiomer and so approximately twice (1.6-fold) the potency of the racemic mixture [20]. However, whilst the *R*-(-)-ketamine enantiomer is significantly less biologically active as an anaesthetic, it is not more toxic, and therefore using the racemic mixture clinically does not give rise to more side-effects. In the UK, ketamine is available as racemic mixtures [20] while a single enantiomer product is usually imported. In this case, the required pain relief effect can be achieved with a much lower dosage (of one enantiomer) and a quicker recovery time from the anaesthetic [23]. The production of ketamine by Pfizer Ltd, the only provider in the UK, has been discontinued (June 2014), and so other sources might be a potential source of *S*-ketamine, e.g. as Ketanest[®]-*S*. Therefore, there is a chance to import *S*-ketamine [24].

The dose of ketamine is varied between individual patients depending on bodyweight and route of administration. Analgesia can be produced by intravenous injection of 0.2-0.75 mg/kg. If ketamine is injected directly into a vein at 2 mg/kg, this can cause surgical anaesthesia within 30 s and that effect will last for 5-10 min. While injecting 10 mg for every kg bodyweight into a muscle can last longer, from 12 to 25 min with onset within 3 to 4 mins [25]. Psychotropic effects are also produced by intramuscular (i.m.) injection of high dose of ketamine, between 25 to 200 mg in humans [26]. Generally, low doses of ketamine can cause dreamy thinking, euphoria, and mild hallucinations with separation of time and space. At large doses, ketamine can make people physically incapable of moving and

completely detached from their body and surroundings. This is often called a “near-death experience” or “entering the k-hole” [27]. Abusers attempt to evaporate ketamine solution to form crystals and then grind the residue into a powder before inhaling it or mixing it with alcohol [28], or the liquid can be injected i.m. [1]. Abusers are reported to snort between 60 and 250 mg at a time [15]. Oral administration is rare; ketamine is metabolized to norketamine (*N*-demethylation), which produces more sedation and is less hallucinogenic [1].

Ketamine is produced commercially by various pharmaceutical companies. It was first made by Stevens in 1962, Belgian Patent 634208 (1963). The synthesis of the optical isomers is also described by Hudyma et al., German patent 2062620 (1971 to Bristol-Myers) [29]. Nevertheless, the absolute stereo-structures of *S*- and *R*-ketamine hydrochloride were first described by Ratti-Moberg et al. in 1991 by using tartaric acids as resolving agents [30]. Other syntheses of ketamine and analogues are reported [31, 32] including those employing thermal re-arrangement [33].

Ketamine is a prescribed drug and reported to be difficult to synthesise which partly explains why it is often stolen from pharmaceutical companies, veterinary practices, and hospitals as happened in Mexico and France [20, 34]. The Advisory Council on the Misuse of Drugs (ACMD) reported that “Data on the diversion of ketamine from human or veterinary practice are not collected nationally. However, there have been reports from Controlled Drug Local Intelligence Networks that have evidence of diversion of ketamine from health and veterinary settings in some localities” [35]. In recent times, the illicit production of ketamine has been shown to occur in China on a village-wide 0.5 ton scale in Boshe [36]. Clandestine ketamine laboratories are also reported in India, Indonesia, and the Philippines [37].

To the best of our knowledge, there has not been any study to investigate the sources of ketamine within the UK using impurity profiling and preservative detection of street and legal ketamine samples by combining NMR and ESI-MS. A specific analytical method has therefore been developed by using ¹H NMR to detect preservative as low as 30 µg/mL in legal and illicit ketamine. The information obtained from this investigation can be used to aid law enforcement in tracking the diversion of ketamine from legal sources and/or illegal production to establish drug networks and distribution patterns.

6.2. Experimental

6.2.1. Materials



Fig. 2. Street ketamine sample forms and ketamine powder inhalers from Glastonbury and a Bristol night club, 2013

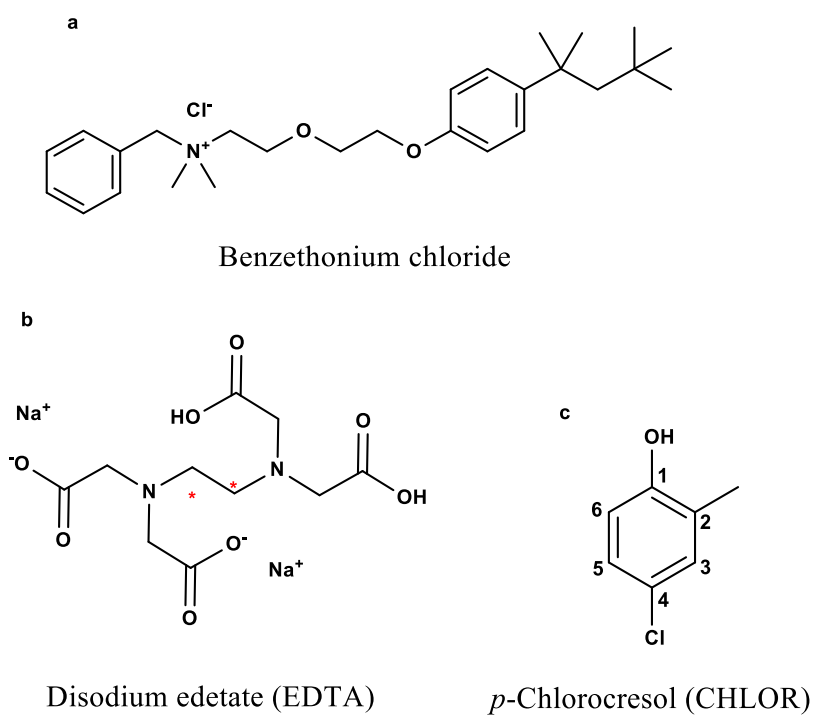


Fig. 3. Preservatives present in ketamine hospital and veterinary formulations available in the UK, (a) BZC, (b) EDTA, and (c) *p*-chlorocresol

Ketamine samples (59) were supplied by the police from amnesty bins provided in a Bristol UK night club and the Glastonbury music festival (both in 2013) as well as 5 ketamine samples seized from two different places in Bristol.

Street ketamine samples were received as crystalline needles and powders of different colours. These samples were received in different amounts in small plastic bags and two samples came in inhalers with ketamine placed inside them (Fig. 2).

Two different batches of ketamine hydrochloride (KT HCl) (Lot #SLBH9550V and Lot #SLB4519V, India), D₂O (99.8 atom % D), and all other solvents of HPLC grade ($\geq 99.9\%$ purity) were purchased from Sigma Aldrich, UK. All preservatives and antioxidants within the pharmaceutical formulations of ketamine in the UK were purchased from Sigma Aldrich (UK) as reference materials: *p*-chlorocresol (4-chloro-2-methylphenol, preservative and antiseptic, 99.8%); ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA disodium salt, antioxidant, 98.5-101.5%), and benzethonium chloride (BZC, preservative, $\geq 98\%$ HPLC) (Fig. 3). Hospital and veterinary ketamine, used in the UK, were purchased as colourless liquid solutions in vials ready for injection, pictures of KetalarTM and VetalarTMV are shown in Fig. 4.



Fig. 4. Hospital and veterinary ketamine in vials ready for injection.

Names of hospital and veterinary ketamine medicinal products together with their formulations' content and suppliers are given in Table 1. All of these are available as clear solutions in vials ready for injection [21, 22]. Based on the VMD brand name, Anesketin 100 mg/mL solution for injection for dogs, cats and horses is produced in Denmark, France, Germany, Netherlands, Spain, and the UK. The same formulation Nimatek 100 mg/mL solution for injection for dogs, cats and horses is manufactured in Austria, Belgium, Ireland, Italy, Poland, Portugal, and the UK. The veterinary ketamine products information in Table 1 was updated on 04.02.16 [22].

Table 1

Summary of veterinary and hospital ketamine product characteristics and their suppliers in the UK

		<i>Brand name</i>	<i>Active substance mg/mL</i>	<i>KT HCl, mg/mL</i>	<i>preservative</i>	<i>Manufacturer</i>
Veterinary (VMD)	1	Anaestamine [®]	100	115.33	CHLOR (1 mg/mL)	Animalcare
	2	Anesketin	100	115.4	CHLOR (1 mg/mL)	Dechra
	3	Ketavet [®]	100	115.36	BZC (0.10 mg/mL)	Zoetis UK Ltd
	4	Narketan [®] -10	100	100	BZC (0.10 mg/mL) and EDTA (0.1 mg/mL)	Vétoquinol
	5	Ketamidol [®]	100	100	BZC (0.10 mg/mL)	Chanelle
	6	Vetalar [™] V	100	100	BZC (0.01% w/v)	Pfizer
	7	Nimatek*	100		CHLOR (1 mg/mL)	Eurovet Animal Health B.V.
Hospital (BNF)	1	Ketalar [™]	50	50	BZC (1:10000)	Pfizer

Nimatek is discontinued; Ketaset[®] is the same as ketavet[®];

CHLOR: *p*-chlorocresol; BZC: benzethonium chloride;

EDTA: ethylenediaminetetraacetic acid disodium salt dihydrate

6.2.2. Instrumentation and experimental procedures

6.2.2.1. General procedure

The crystalline and powder in the seized and amnesty bins ketamine samples were labelled after being identified by ¹H NMR and confirmed by ESI-MS. Each sample was ground into a powder. The hospital and veterinary samples of ketamine were poured on to glass plates and allowed to evaporate in a fume cupboard for 2-3 h. The resulting crystals were dried under a stream of anhydrous nitrogen, ground, labelled, and stored in a safe at 18 °C. The analytical balance was a Sartorius Microbalance SE-2F, precision 0.1 µg; Sartorius AG, Goettingen, Germany. UV spectra were recorded on a Perkin Elmer-Lambda 35-UV/Vis Spectrometer.

6.2.2.2. LC-ESI-MS analysis

Positive and negative ion modes were reported on a liquid chromatography mass spectrometer (LC/MS, a Bruker Daltonics micrOTOFmass spectrometer) fitted with an electrospray ionisation (ESI) source. The samples were dissolved in methanol (~10 µg/mL) and ketamine, their impurities and preservatives were detected.

6.2.2.3. NMR analysis

The 1D and 2D NMR experiments ^1H , ^{13}C HSQC, HMBC, H2BC, DEPT, and ^{15}N -HMBC were performed on the 500 MHz spectrometer. ^1H NMR were measured for the ketamine samples in D_2O (50 mg/mL) on a Bruker (500 MHz) spectrometer. The pharmaceutical formulations of ketamine contained preservative, antiseptic, and antioxidant ingredients alongside the active component, ketamine hydrochloride (Table 1). The experiment has been optimized to be detect: benzethonium chloride at 5 µg/mL and 50 µg/mL, EDTA at 50 µg/mL, and *p*-chlorocresol at 500 µg/mL. Full NMR assignment is also reported for the authentic ketamine and each preservative. Each spectrum was referenced and rescaled by adjusting the residual HOD signal (in D_2O) to 4.79 ppm. All spectra have been processed using Topspin 1.3, 2.0, or 2.1 program suite (Bruker Biospin GmbH, Rheinstetten, Germany) and MestReNova (Version 10.0.2).

6.2.2.4. Optical activity of ketamine samples

The optical profiling for all ketamine samples was tested on an ADP-220 polarimeter (Bellingham and Stanley, Tunbridge Wells, UK). Each ketamine sample was prepared in MeOH (1 mg/mL). In each measurement, a sample was placed in a 1 mL cell and measured after subtracting the background of the solvent, MeOH. If any of the street ketamine samples has predominant or even a single enantiomer *S*, this is evidence that the sample is not necessarily an illegal synthetic ketamine, but it might possibly be a hospital ketamine that has been imported.

6.2.2.5. Isotope profiling of ketamine samples

Table 2

Identification of KT samples by origin

<i>Sample</i>	<i>Country of origin</i>	<i>Supplier</i>
<i>VetalarTM V</i>	Germany	Zoetis UK Ltd
<i>KetalarTM</i>	Ireland	Pfizer Ltd, Kent, UK
<i>Ketamine HCl</i>	India	Sigma Aldrich (authentic)
<i>GB2-1, GB3-2, GB4-1, GA7, GA11, GB14-11</i>	Glastonbury music festival amnesty bin (2013)	Avon and Somerset Constabulary, UK
<i>AH9, AS5, AV1</i>	A night club in Bristol, UK (2013)	Avon and Somerset Constabulary, UK
<i>K2, K3</i>	Seized samples, Avon and Somerset Constabulary	Drug Expert Action Team (DEAT), UK

Eleven different ketamine street samples, together with three authentic samples (Table 2) (including hospital Ketalar and veterinary Vetalar), were subjected to Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) analysis.

The %C and %N elemental compositions were determined by elemental analyser (Dumas combustion). The analysis was carried out on a Carlo Erba NA1500 Mk 1 elemental analyser coupled to a PDZ Europa CF-IRMS. In this technique, the sample is combusted, the gaseous products separated by GC, and the peak area of each determined by thermal conductivity. The system is calibrated using primary (NIST) standards of known % N and C. All the stable isotope ratios δ (‰) and % element data are traceable to the National Institute of Standards and Technology (NIST) primary references. δ (‰) ^{15}N calibrated to IAEA-N1 ammonium sulfate and ^{13}C calibrated to reference IAEA-CH-3 cellulose. Sample (2.0-2.5 mg) of the ketamine samples were accurately weighed and submitted in tin capsules for EA-IRMS. The δ values are subject to various correction factors, but in essence they are derived from the following equation:

$$\delta(\text{‰}) = [(R_{\text{Sample}}/R_{\text{Std}}) - 1] \times 1000 \quad (1)$$

where: $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio, and R_{Std} ratio of the international standard

6.2.2.6. Single-crystal X-ray diffraction

X-Ray diffraction was measured for one of the seized ketamine samples that was confirmed to have no optical activity by polarimetry. The crystals were formed from drops of methanol and hot cyclohexane and left for 3 days at 18 °C. The crystals were collected, and submitted for X-ray diffraction analysis (University of Bath).

6.3. Results and discussion

6.3.1. NMR assignments of ketamine and their pharmaceutical preservatives

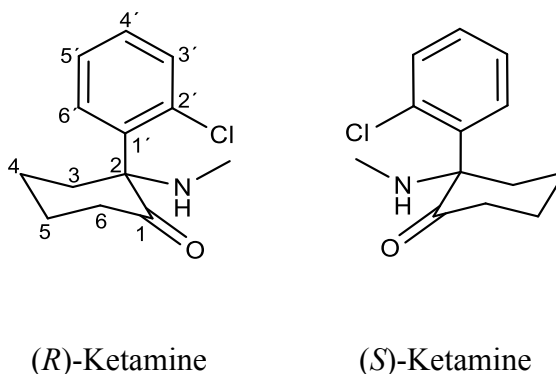


Fig. 5. Optical isomers of ketamine

Legal ketamine is available as ketamine hydrochloride solution ready for injection in different formulations and concentrations (Table 1). Medical ketamine in the UK is available as the racemic mixture (Fig. 5). The molecular structure of ketamine (authentic) as well as its preservatives and antioxidant were confirmed by NMR. The ^1H and ^{13}C chemical shifts in D_2O were assigned using COSY, HSQC, HMBC, ^{15}N HMBC and H2BC experiments. The data also show good agreement with the literature [38]. ^1H NMR (500 MHz, D_2O) δ 1.64-1.77 (m, 2H, $\text{H}_{4\text{eq}}$, $\text{H}_{5\text{eq}}$), 1.84-1.91 (m, 2H, $\text{H}_{3\text{eq}}$, $\text{H}_{4\text{ax}}$), 2.04-2.11 (m, 1H, $\text{H}_{5\text{ax}}$), 2.37 (s, 3H, N-Me), 2.50-2.61 (m, 2H, $\text{H}_{6\text{ax}}$, $\text{H}_{6\text{eq}}$), 3.26-3.33 (m, 1H, $\text{H}_{3\text{ax}}$), 7.52-7.59 (m, 3H, in the ascending order $\text{H}_{5'}$ then $\text{H}_{4'}$ then $\text{H}_{3'}$ following from HSQC), 7.83 (dd, $J = 7.0, 2.0$ Hz, 1H, $\text{H}_{6'}$). ^{13}C (500 MHz, D_2O) δ 21.0 (C4), 26.6 (C-N-Me), 30.0 (C5), 35.9 (C3), 39.3 (C6), 72.4 (C2), 127.0 (C1'), 128.3 (C4'), 131.7 (C6'), 131.8 (C3'), 132.6 (C5'), 133.9 (C2'), 211.2 (C1) (Fig. 6). Exact mass ion patterns of ketamine, $\text{C}_{13}\text{H}_{17}\text{ClNO}$ requires 238.0993 found 238.0982 (4.6218 ppm), are shown in Fig. 7.

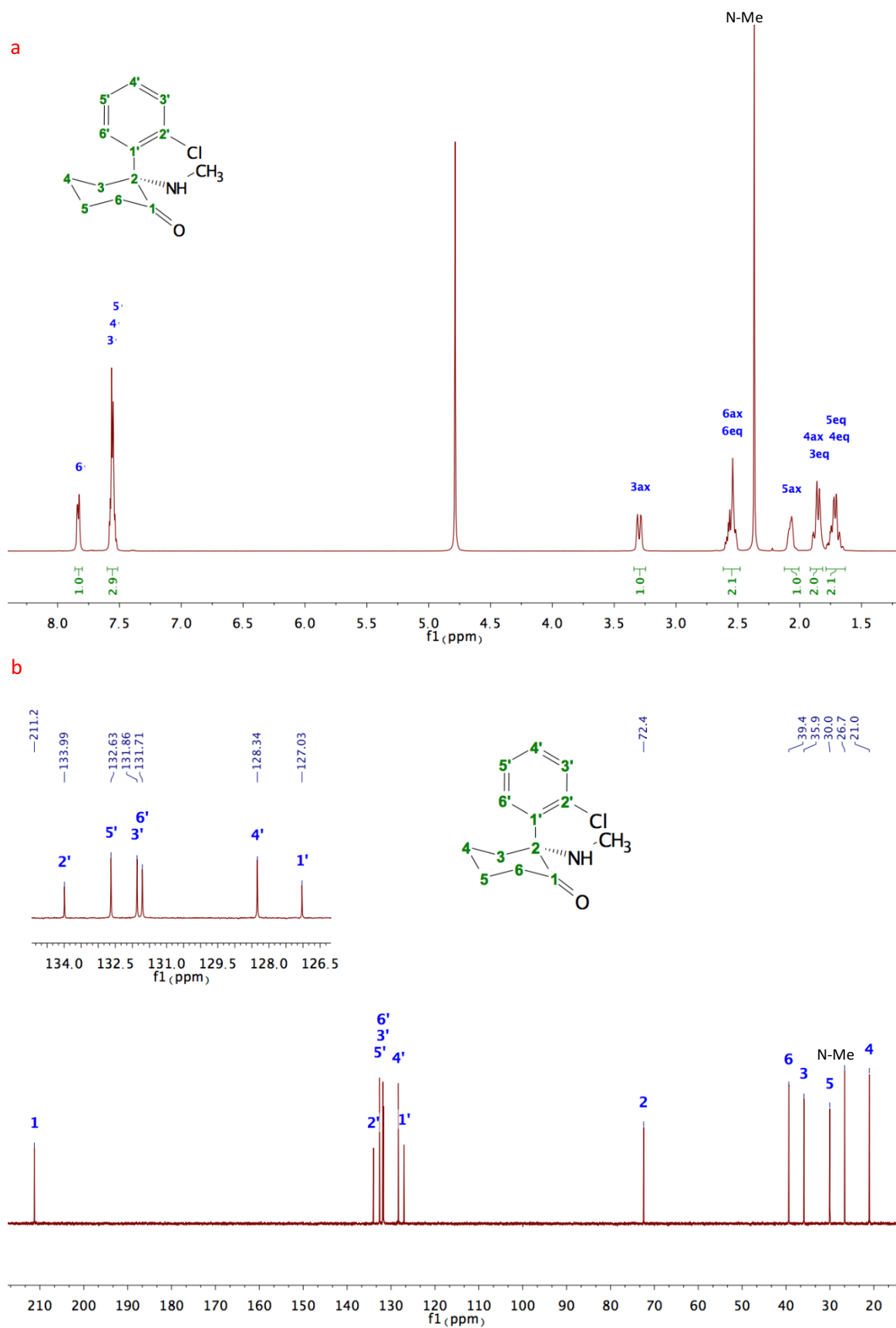


Fig. 6. (a) ^1H and (b) ^{13}C NMR assignments of an authentic ketamine in D_2O

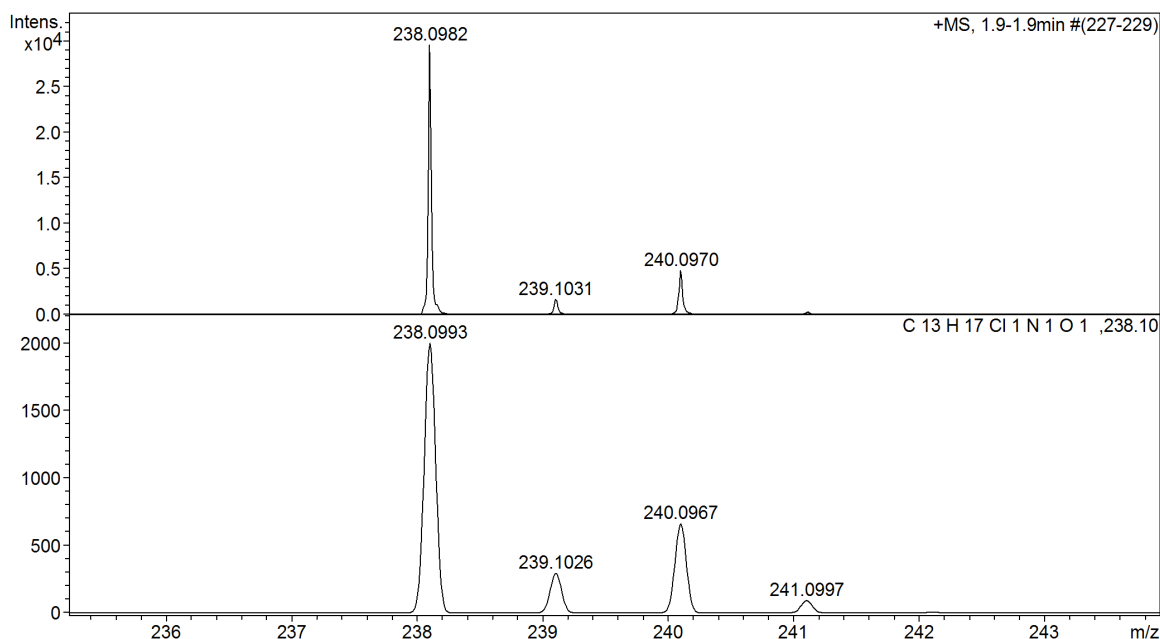


Fig. 7. MS for ketamine and the simulated pattern for the formula C₁₃H₁₇ClNO showing the 3:1 ³⁵Cl:³⁷Cl isotope ratio

The aromatic proton peak pattern for ketamine clearly shows a 1,2-disubstituted benzene pattern: multiplet (7.55 ppm, 3H), a doublet (7.83 ppm, 1H) (Fig. 6 (a)). The ¹³C NMR spectrum of ketamine contained 13 carbons, four of them are quaternary as no signals are detected in HSQC, one aliphatic quaternary (C2, 72.4 ppm), two aromatic quaternary (C1' = 127.0 and C2' = 133.9 ppm) and one ketone (C1 = 211.2 ppm) (Fig. 6 (b)). The HSQC spectrum shows the correlation between each carbon and its corresponding proton. Two protons from different carbons are responsible for each of the two first peaks around 1.70 and 1.88 ppm (Fig. 8), these were assigned later by HMBC as 4_{eq}, 5_{eq}, and 3_{eq}, 4_{ax}, respectively (Fig. 10). In the dihedral angle in cyclohexanone, spin-spin coupling can occur through 2, 3, and 4 bonds. Axial protons typically resonate 0.5-1.5 ppm downfield from equatorial ones, and not all proton signals are split by their close protons. The NH proton in NH-Me is exchangeable due to its acidity, therefore usually no coupling will occur between this and other protons. The presence of the *N*-Me is observed by ¹H 2.37 ppm, ¹³C 26.6 ppm, COSY, HSQC, and confirmed by a ¹⁵N HMBC NMR experiment. The correlation between the nitrogen atom and the methyl group is illustrated in Fig. 9.

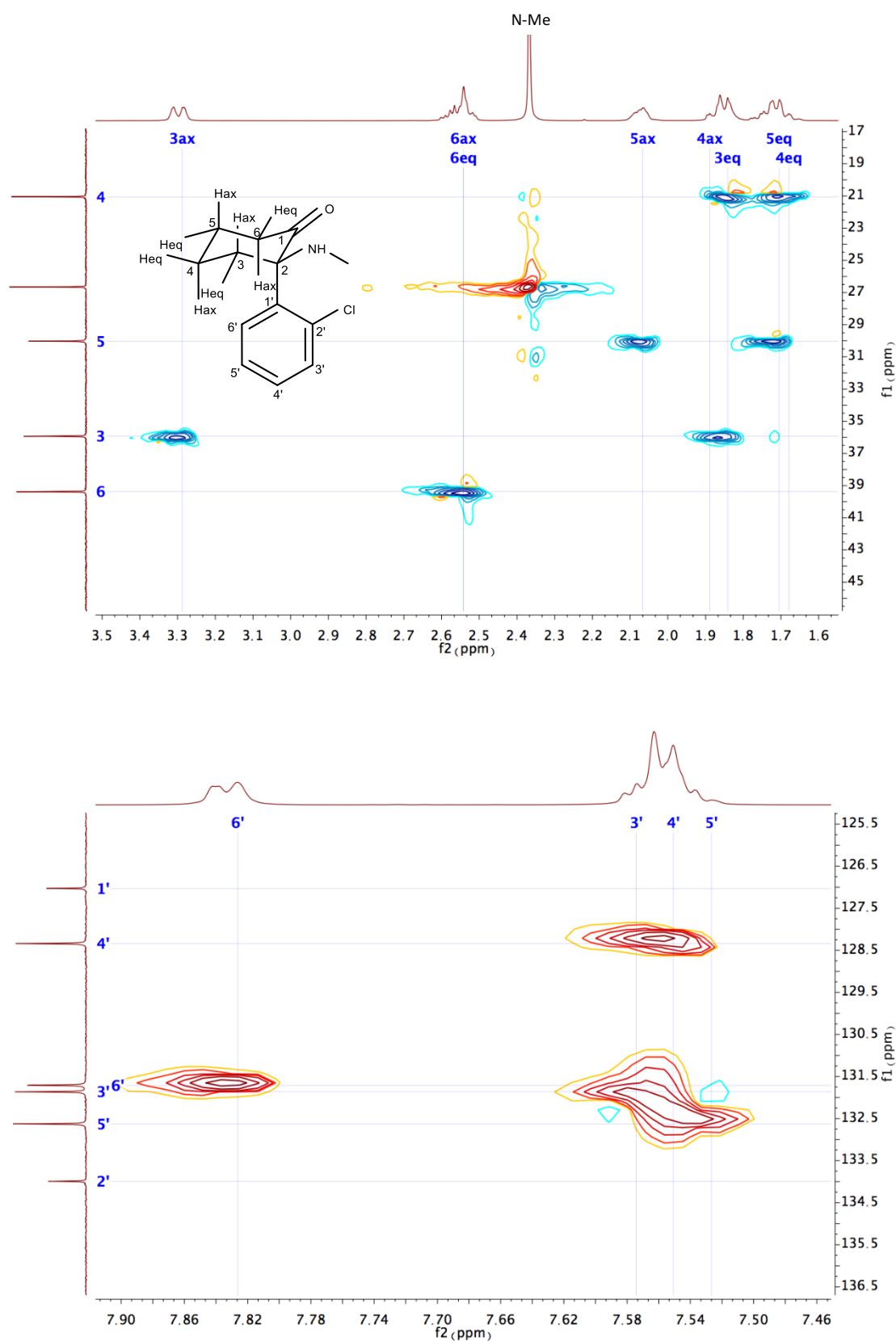


Fig. 8. HSQC assignments of authentic ketamine

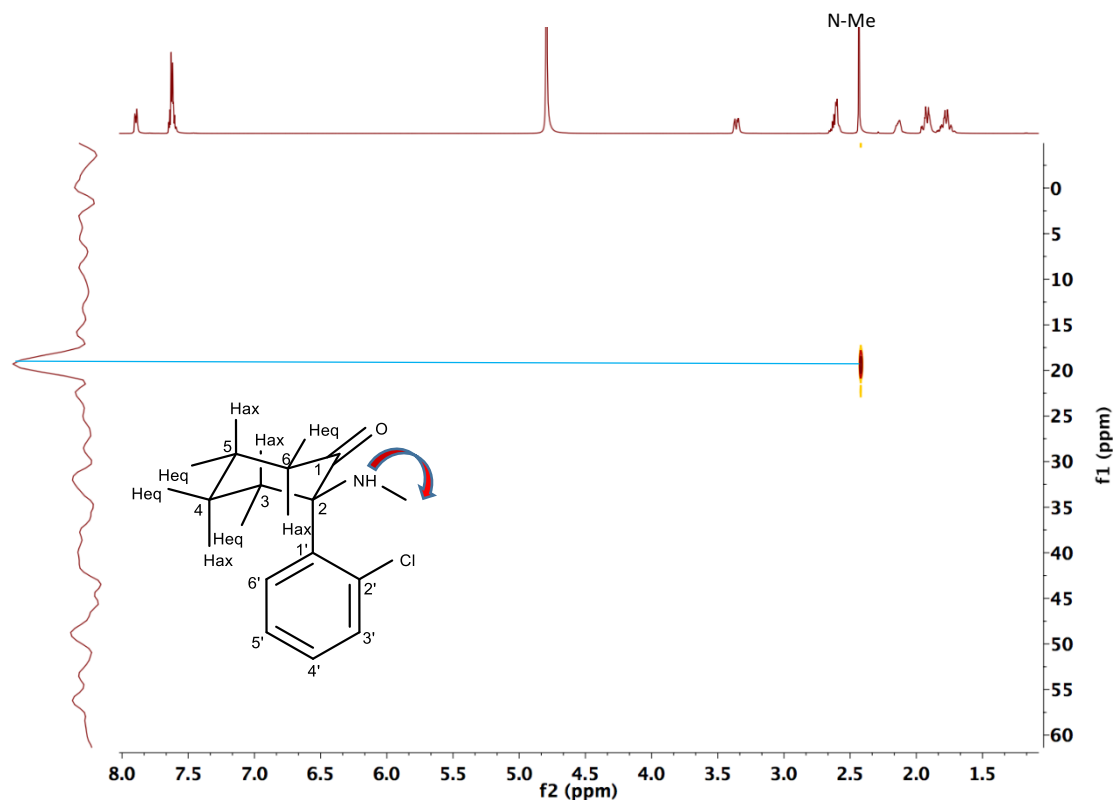


Fig. 9. ^{15}N -HMBC NMR experiment of ketamine in D_2O showing the correlation between the *N*-atom and the singlet at 2.38 ppm, 3H, confirming the presence of the *N*-methyl group

Starting with the quaternary signals, the HMBC (Fig. 10) shows correlations between the aromatic quaternary $\text{C}1'$ with $6'$, $5'$, and the 3 equatorial (eq) and axial (ax) protons at 1.84-1.91 (m) and 3.30 (dd) ppm, respectively. These correlations show that $\text{C}1'$ is bonded to, or very nearby, the $6'$, $5'$ aromatic carbons and carbon-3 in the cyclohexanone. In addition, the carbonyl carbon at 211 ppm is coupled with $\text{H}3_{\text{ax}}$, $\text{H}6_{\text{ax}}$, $\text{H}6_{\text{eq}}$, and the nearby proton of $\text{C}5$, $\text{H}5_{\text{ax}}$. The quaternary carbon at 72.5 ppm is identified as $\text{C}2$ as the only aliphatic quaternary besides the carbonyl. The chemical shift indicates it is bonded to a strong electron withdrawing group, in this case to the cyclic ketone, the *N*-atom, and the aromatic ring. $\text{C}2$ correlates with $\text{H}3_{\text{ax}}$, $\text{H}6_{\text{ax}}$, $\text{H}6_{\text{eq}}$, *N*-Me, and $\text{H}3_{\text{eq}}$. All other HMBC assignments are illustrated in Fig. 10 and described in Table 3.

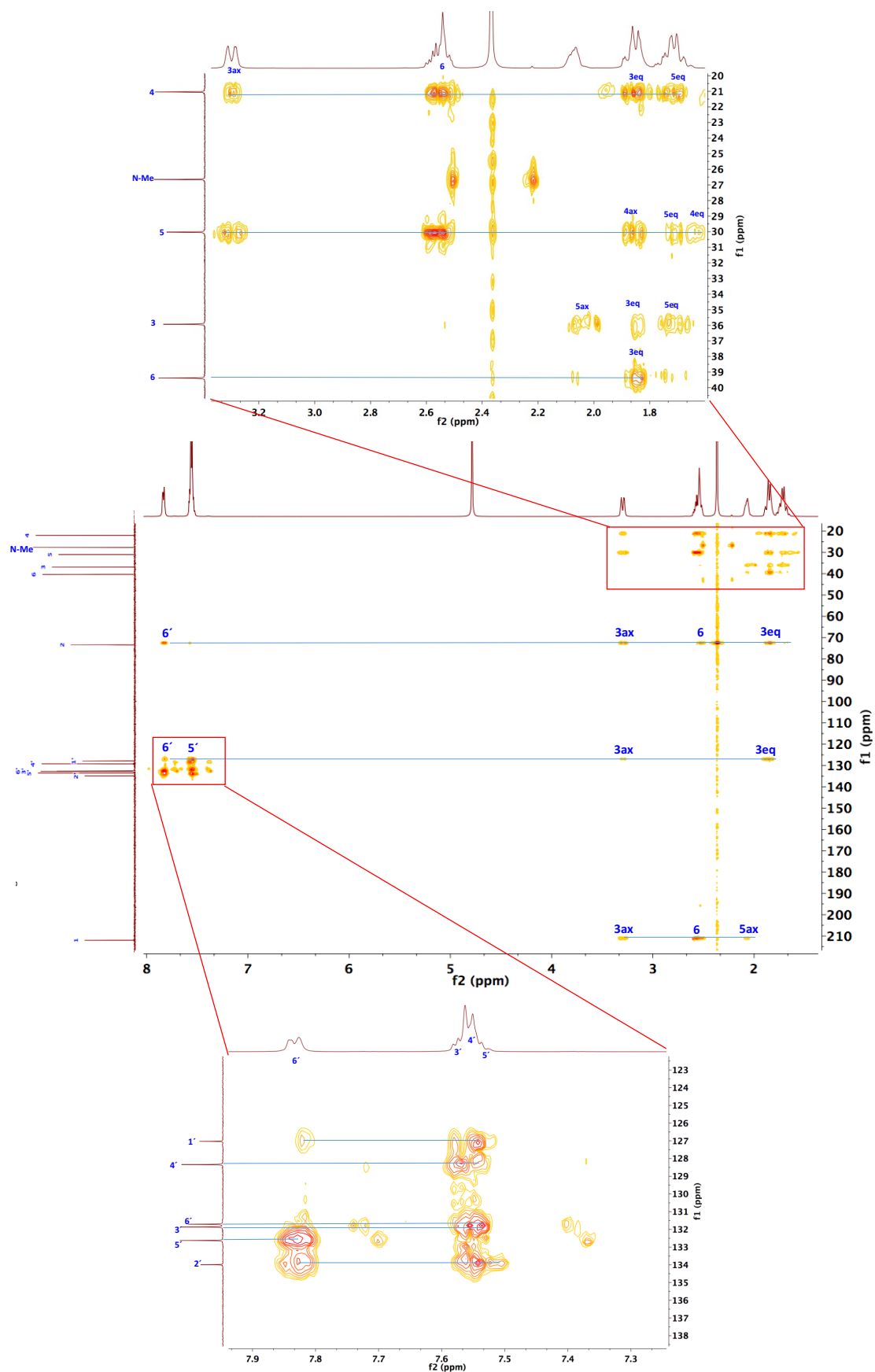


Fig. 10. HMBC assignments of an authentic ketamine sample

Table 3Assignments of ^1H and ^{13}C NMR chemical shifts of ketamine

Position	^{13}C (ppm)	^1H (ppm, J) and HSQC	HMBC
1	211.2	-	3_{ax} , 6_{ax} , 6_{eq} , 5_{ax}
2	72.4	-	$6'$, 3_{ax} , N-Me, 3_{eq}
3	35.9	1.84-1.91 (m, $\text{H}3_{\text{eq}}$, 1H), 3.30 (dd, J = 14.0, 3 Hz, $\text{H}3_{\text{ax}}$, 1H)	5_{ax} , 5_{eq} , 3_{ax} , 3_{eq}
4	21.0	1.64-1.77 (m, $\text{H}4_{\text{eq}}$, 1H), 1.84-1.91 (m, $\text{H}4_{\text{ax}}$, 1H),	3_{ax} , 3_{eq} , 5_{eq} , 6_{ax} , 6_{eq}
5	30.0	1.64-1.77 (m, $\text{H}5_{\text{eq}}$, 1H), 2.04-2.11 (m, 1H, $\text{H}5_{\text{ax}}$)	4_{ax} , 4_{eq} , 3_{ax} , 5_{eq} , 6_{ax} , 6_{eq}
6	39.3	2.50-2.61 (m, 2H, $\text{H}6_{\text{eq}}$, $\text{H}6_{\text{ax}}$)	3_{eq}
$1'$	127.0	-	3_{eq} , 3_{ax} , $6'$, $5'$
$2'$	133.9	-	$3'$, $4'$, $5'$, $6'$
$3'$	131.8	7.57 (d, J = 2.5 Hz $\text{H}3'$, 1H)	$4'$, $5'$
$4'$	128.3	7.55 (d, J = 2.5 Hz $\text{H}4'$, 1H)	$3'$, $5'$
$5'$	132.6	7.53 (d, J = 2.5 Hz $\text{H}5'$, 1H)	$6'$
$6'$	131.7	7.83 (dd, J = 6.0, 2.5 Hz, 1H)	$4'$, $5'$
N-Me	26.6	2.37 (s, N-Me, 3H),	

Benzethonium chloride ^1H NMR (500 MHz, D_2O) δ 0.6 (s, 9H, 1), 1.21 (s, 6H, 5), 1.50 (s, 2H, 3), 3.01 (s, 6H, 17), 3.41 (s, 2H, 11), 3.82 (s, 2H, 13), 4.01 (s, 4H, 10, 12), 4.53 (s, 2H, 15), 6.75 (d, J = 2.5 Hz, 2H, 7), 7.09 (d, J = 10.0 Hz, 2H, 17), 7.14-7.20 (m, 3H, 18, 19), 7.42 (d, J = 2.5 Hz, 2H, 8) (Fig. 11); λ_{max} = 276 nm. $[\text{M}]^+$ of base $\text{C}_{27}\text{H}_{42}\text{NO}_2$ requires 412.3210 found 412.3224 (3.3980 ppm).

EDTA ^1H NMR (500 MHz, D_2O) δ 3.64 (d, J = 2.5 Hz, 4H), 3.86 (d, J = 2.5 Hz, 8H) (Fig. 12).

p-chlorocresol ^1H NMR (500 MHz, D_2O) δ 2.14 (s, 3H, CH_3), 6.78 (d, J = 10.0 Hz, 1H, 6), 7.11 (dd, J = 10.0, 5.0 Hz, 1H, 5) 7.16 (d, J = 5.0 Hz, 1H), (Fig. 13); λ_{max} = 283 nm; $[\text{M-H}]^-$ $\text{C}_7\text{H}_7\text{ClO}$ requires 141.0112 found 141.0116 (2.8368 ppm).

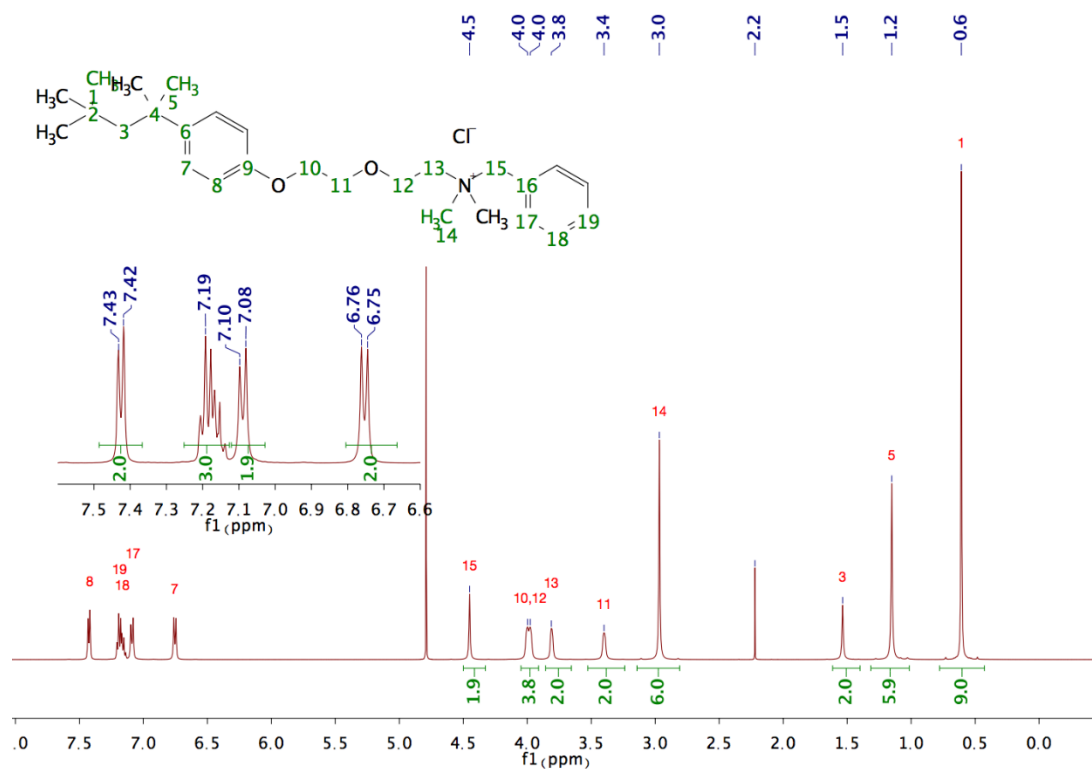


Fig. 11. ^1H NMR assignment of BZC in D_2O with the J coupling values

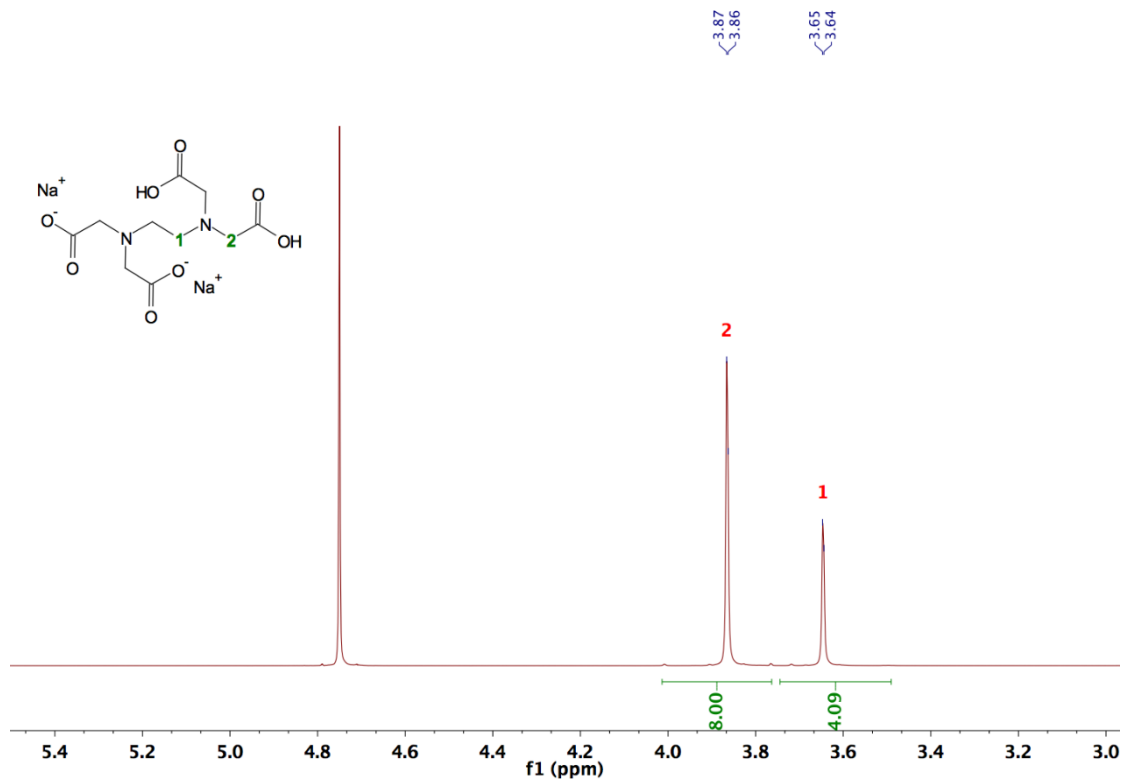


Fig. 12. ^1H NMR assignment of EDTA in D_2O

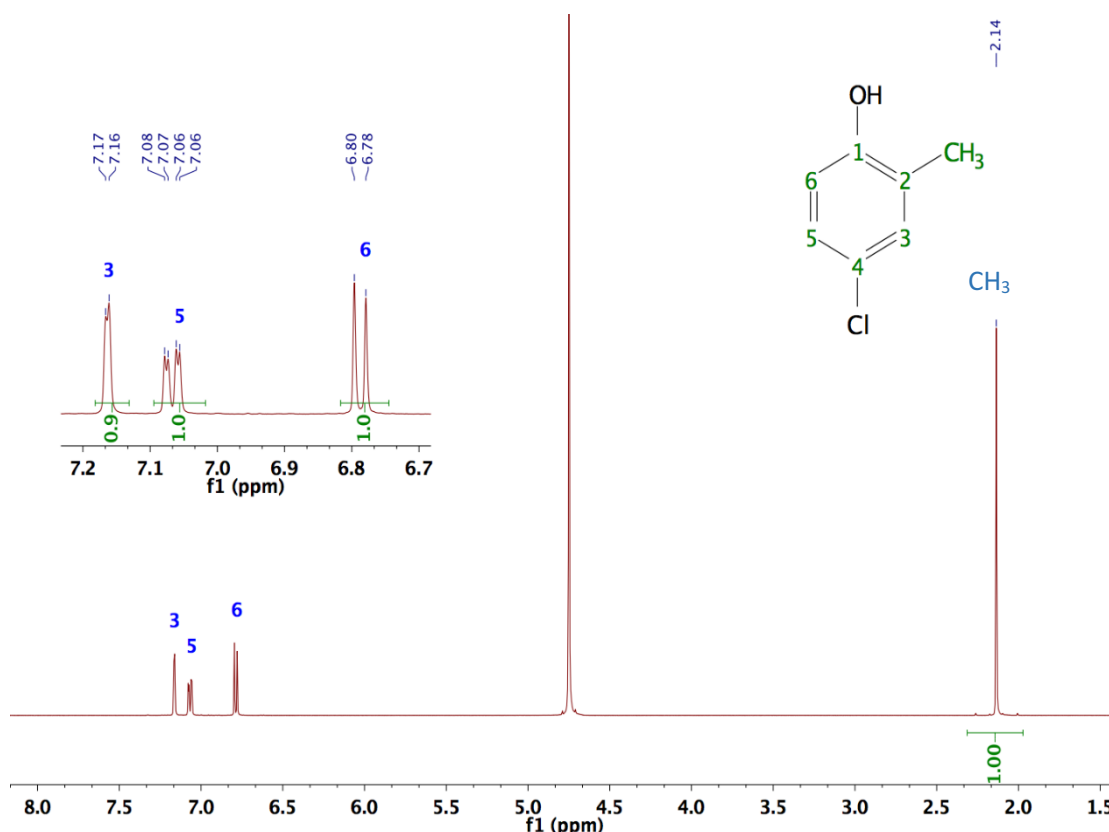


Fig. 13. ^1H NMR (D_2O) assignment of *p*-chlorocresol (aromatic area expanded)

6.3.2. ^1H NMR method validation of ketamine preservatives detection

Standard ^1H NMR (number of scan, $NS = 16$) was run first to identify ketamine within the seized and amnesty bins samples. The second aim was to search if any of the street samples contained pharmaceutical preservatives (BZC, EDTA, and CHLOR), Fig. 14. Based on the impurity profiling of the street ketamine samples by ^1H NMR, most of the samples were preservative free, e.g. sample code AL9, Fig. 15 (a). Nevertheless, MDMA impurity can be identified by increasing the NS from $NS = 16$ to 1024 and expand the spectrum (Fig. 15 (b)). To enhance the sensitivity and to get better signal to noise (S/N), different NS scan were applied on one of the pharmaceutical ketamine samples, VetalarTMV, to determine the NS needed to be able to detect the lowest concentration of preservative BZC at 30 $\mu\text{g/mL}$ (Fig. 16). The $NS = 64$ has been chosen as priority of time vs peaks detection. It can be seen from Fig. 16 that peaks at $\delta = 0.6$ ppm (3CH_3), $\delta = 1.15$ ppm (2CH_3), $\delta = 2.97$ ppm and $\delta = 6.75$ ppm (CH_{arom}) are characteristic for BZC (see the BZC ^1H

NMR assignment Fig. 11) and easily distinguished from the ketamine peaks. The lowest concentration of the preservative, BZC, is present in VetalarTMV (100 mg/mL) at 0.1 mg/mL. Both ketamine and BZC in a VetalarTMV sample are detected in positive ion electrospray with molecular ion $[M]^+$ at m/z 238.0992 (0.4201 ppm) and at 412.3191 (ppm) matching those of a standard of ketamine and benzethonium chloride, respectively.

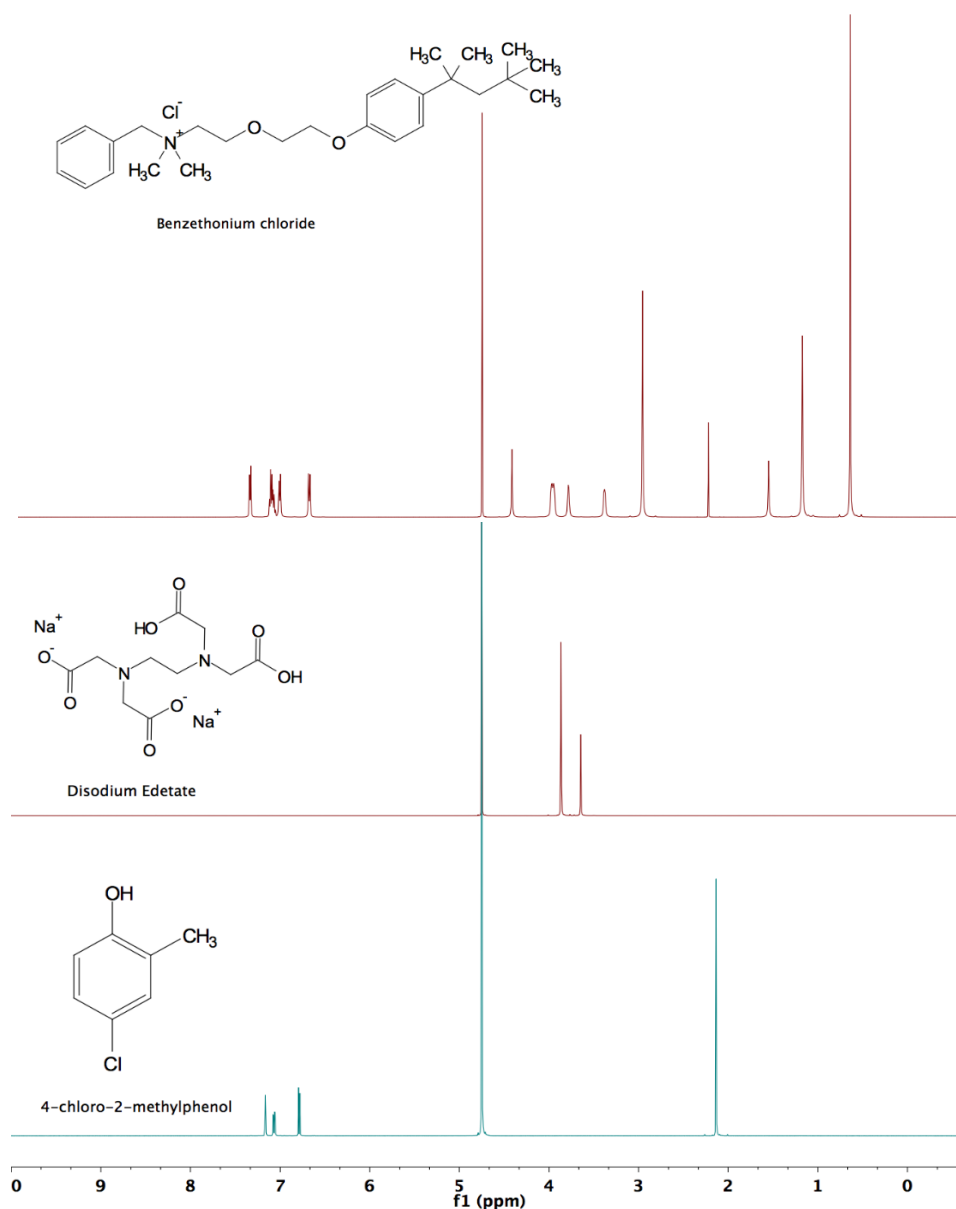


Fig. 14. Stacked spectra of the three preservatives identified within the medical formulations of ketamine: BZC, EDTA, and *p*-chlorocresol (D₂O)

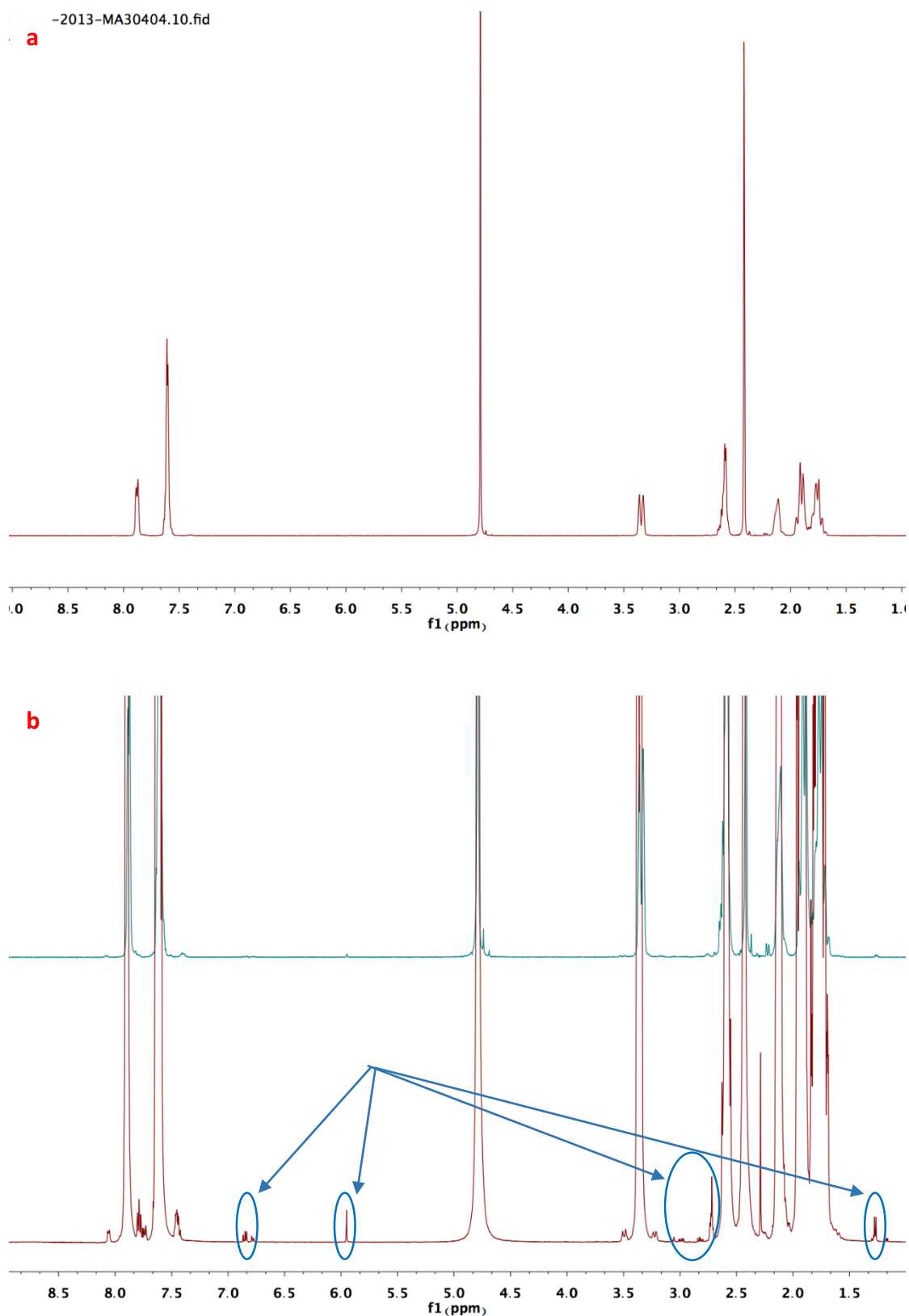


Fig. 15. ^1H NMR spectrum (D_2O) of ketamine (code AL9) showing the differences between (a) ^1H NMR $NS = 16$ and (b) enhanced $NS = 1024$ in detecting any impurity

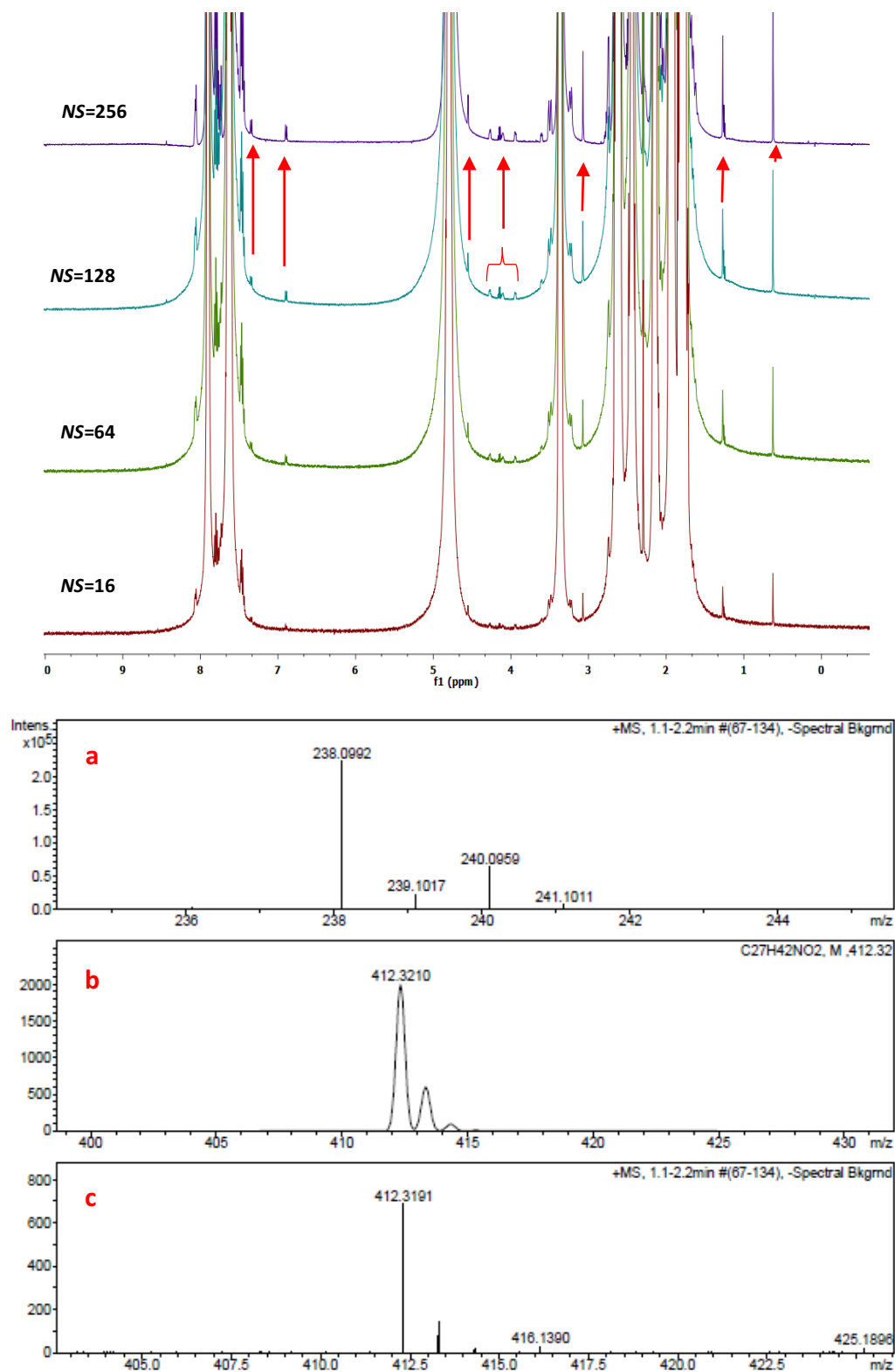


Fig. 16. ^1H NMR of VetalarTMV samples showing how *NS* effects the detection of BZC at a particularly low concentration (30 $\mu\text{g/mL}$), and (a) the exact ion mass of ketamine, (b) simulated mass pattern of BZC, and (c) exact ion mass of BZC in VetalarTMV

^1H NMR spectrum of two medical ketamine samples, Anesketin and Ketamidor were compared with their authentic preservatives, Fig. 17 and Fig. 18 respectively. A shift occurs in the CHLOR and BZC peaks positions. This is due to the presence of ketamine or as a result of a change in pH. An authentic ketamine sample (50 mg/mL, D_2O) spiked with benzethonium chloride (1 mg/mL) (Fig. 19) was prepared to simulate the pharmaceutical formulation of medical ketamine, Ketavet[®], Ketamidor[®], Ketalar[™] and Vetalar[™]V. Stacked ^1H NMR spectrum of the hospital and the veterinary samples show preservative peaks can be detected in the ^1H NMR in D_2O , Fig. 20. Medical ketamine is available two strengths 50 mg/mL for the hospital formulation while all the veterinary samples are in 100 mg/mL concentrations.

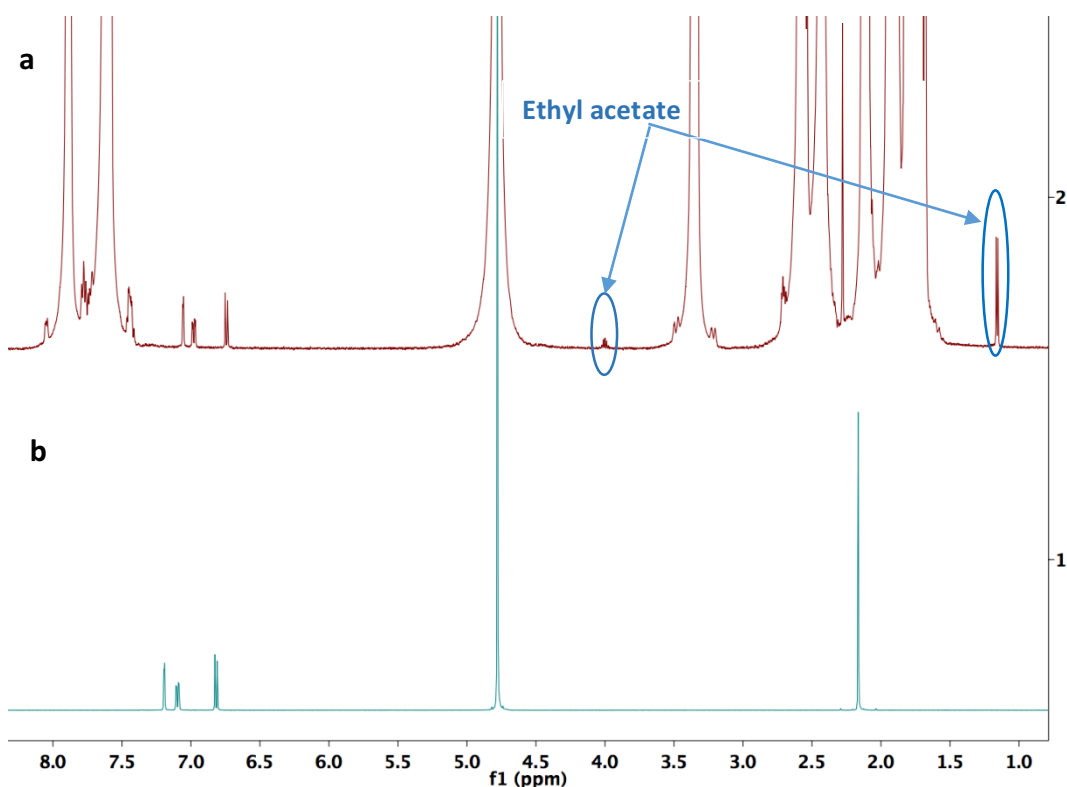


Fig. 17. ^1H NMR (D_2O) spectra of (a) Anesketin (30 mg/mL) containing *p*-chlorocresol (0.3 mg/mL), (b) an authentic *p*-chlorocresol spectrum, and ethyl acetate traces

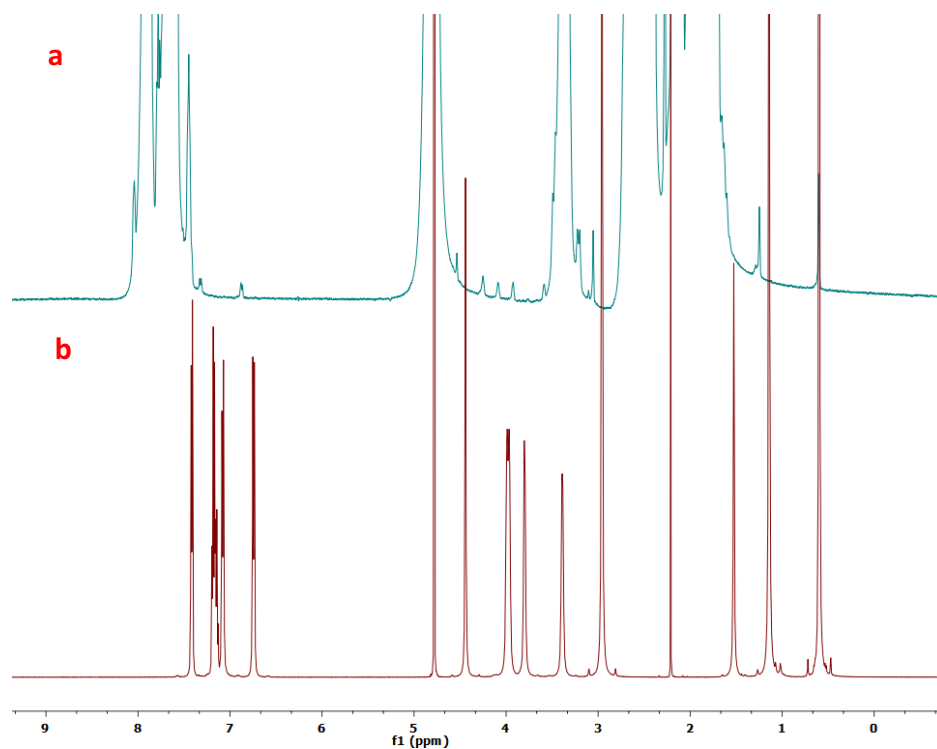


Fig. 18. ^1H NMR stacked spectrum of (a) Ketamidor (30 mg/mL, D_2O) showing the preservative BZC peaks (30 $\mu\text{g/mL}$) are shifted compared with (b) authentic BZC

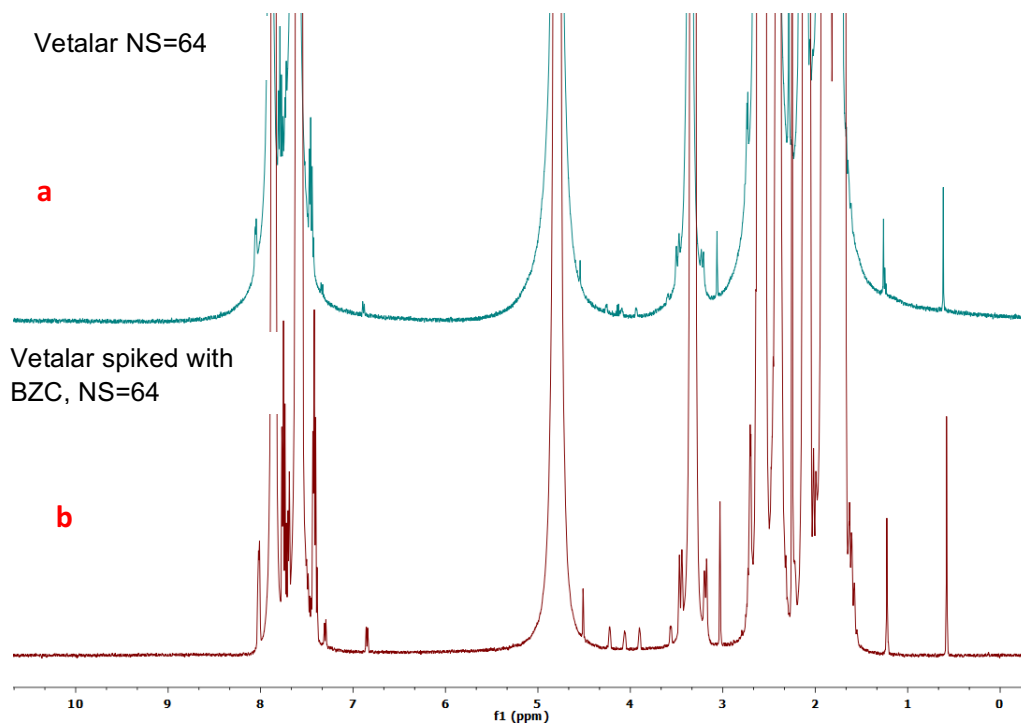


Fig. 19. ^1H NMR of (a) VetalarTMV in D_2O and (b) ketamine spiked with benzethonium chloride

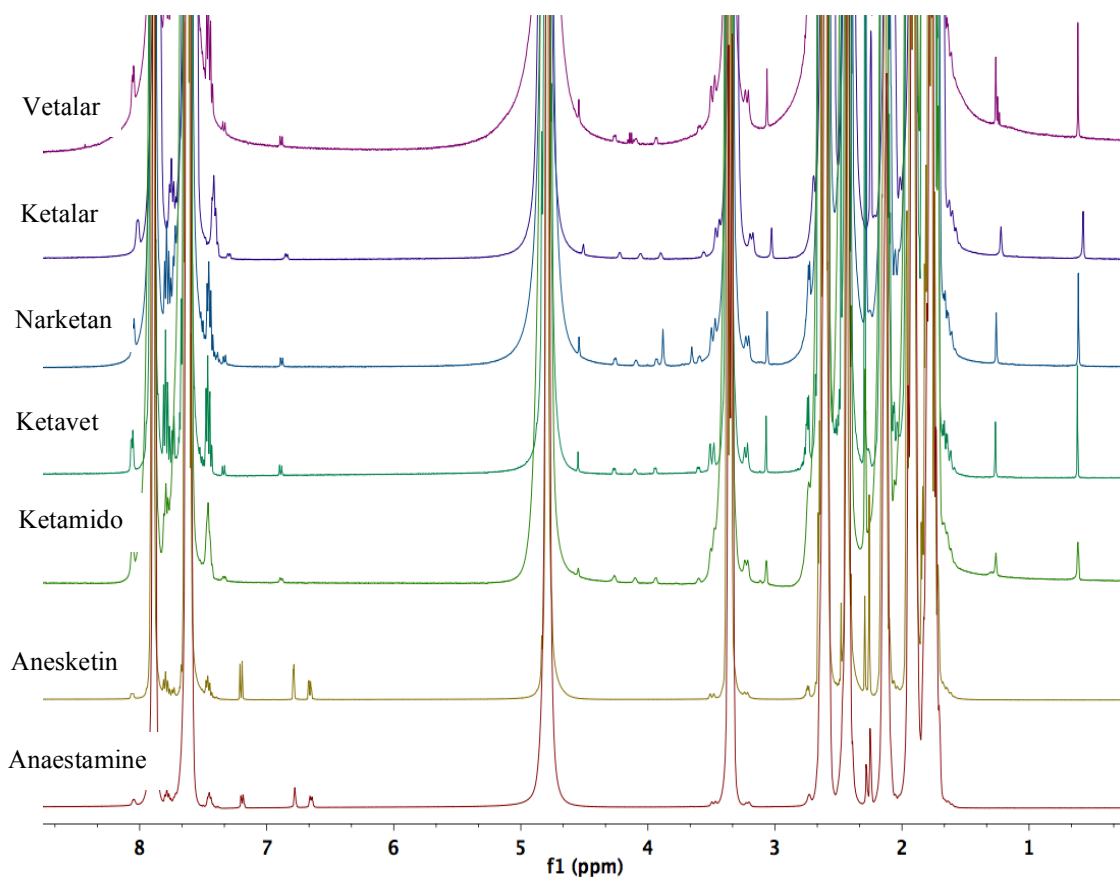


Fig. 20. Stacked ^1H NMR (D_2O) of the hospital and veterinary ketamine formulations that are available in the UK showing expansion in the preservatives areas

6.3.3 ^1H NMR impurity profiling of street ketamine samples

The chemical composition of the ketamine samples was investigated by ^1H NMR and mass spectrometry. The optimized number of scans, $NS = 64$ were used in order to allow for preservative determination. Different NMR experiments were applied for further investigation of the impurities. The ^1H NMR data show that all the street ketamine samples (63) were preservative-free. This indicates that none of the illegal ketamine samples seized and from amnesty bins were diverted from hospitals and veterinary suppliers in the UK. Furthermore, it is likely that the drug being abused is synthetic rather than diverted from B.P. or VMD samples due to the absence of any preservative. Impurity profiling by using ^1H NMR spectroscopy can be used for discriminant analysis, where it is possible to differentiate between samples containing/lacking preservatives.

Although the ketamine samples were preservative-free, other adulterants, solvents and impurities were detected and identified by ^1H NMR. A summary of the adulterants and fillers that were identified in the street ketamine samples is shown in Fig. 21. No evidence was found of the presence of synthetic precursors in the ketamine samples. However, solvents and contaminants were detected and the analysis of individual samples will be discussed below in detail. Impurities are more likely to be precursors or solvents introduced in to the samples during production. While, adulterants are commonly found in illicit drugs and are added at any stage of drug distribution to increase profit. Ketamine is reported to be mixed or cut with other drugs such as methamphetamine, MDMA (ecstasy), cocaine, heroin, and benzodiazepines [39]. Many studies have cited ecstasy tablets cut with ketamine that might be sold as ketamine [15, 28, 34, 40]. Ketamine in tablets is usually sold as ecstasy [28]. Other drugs were that detected in ketamine tablets are methamphetamine, amphetamine, pseudoephedrine, ephedrine, caffeine, and MDMA [28]. Other investigations have reported that ketamine is normally pure and not adulterated and this might be because the drug is diverted from legal sources, relatively new or/and initially used as an adulterant of other drugs [39].

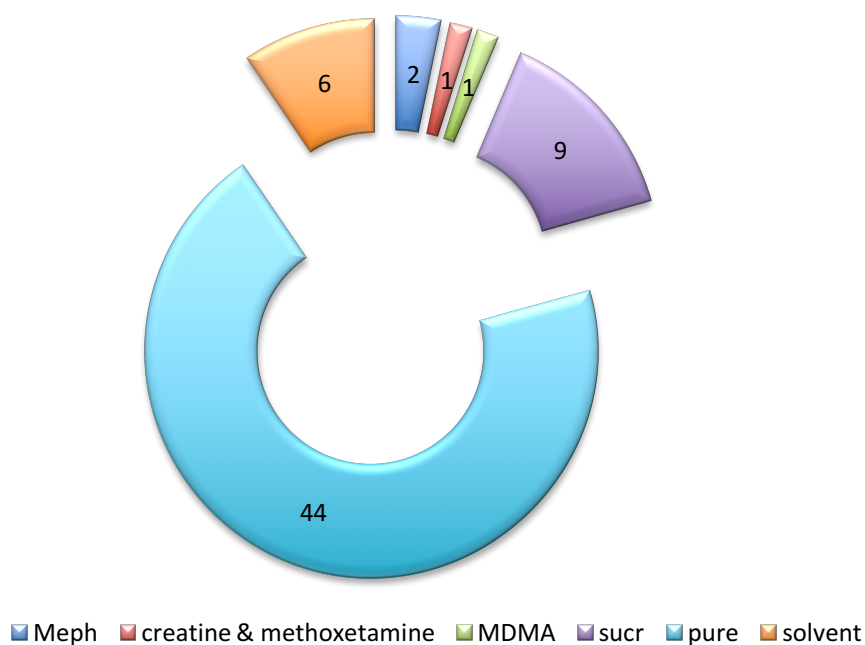


Fig. 21. Number of adulterants and fillers identified in the street ketamine samples

44 samples out of 63 of the illicit ketamine have found to be pure by NMR, purity $\geq 95\%$, Fig. 21. Mephedrone was detected in 3% of the samples. ^1H NMR assignment of mephedrone, and confirmatory mass spectra are displayed in Fig. 22. Mephedrone and ketamine are also used as adulterants in MDMA, 2% (one sample) of the ketamine samples contained MDMA. The samples were compared with authentic MDMA and ketamine ^1H NMR spectra to confirm their identity, see Fig. 23. Sucrose as a diluent is a safe choice for those cutting the ketamine. Sucrose crystals are similar in weight, as heavy as ketamine crystals, easily obtained and not harmful to users, and discussed as being soft (not abrasive) for snorting ketamine. Such aspects of sucrose being a safe choice are found e.g. on [www/bluelight](http://www.bluelight.org) blogs.

One sample of ketamine from the Glastonbury amnesty bin (GG-4) was adulterated with creatine and methoxetamine (MXE). ^1H NMR confirmed by comparing the spectra with the literature [38]. MXE is a designer psychoactive substance chemically related to ketamine and PCP, the dissociative anaesthetics. MXE chemical structure is compared to ketamine in Fig. 24, the 2-chloro and the *N*-methylamine groups are replaced by a 3-methoxy and an *N*-ethylamine group [41]. According to the Advisory Council on the Misuse of Drugs (ACMD) and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), MXE was first advertised on the internet into the UK market in September 2010 as a legal alternative of ketamine [42]. From February 2013, MXE was made illegal in the UK. Yet, MXE is not internationally controlled and remains legal in Brazil, Japan, Russia, Germany, France, and in eight states in the US. Until now, there are no reports on MXE availability and distribution [41].

Ketamine has been discovered to cause serious bladder damage in long-term heavy users [43, 44]. MXE was introduced as a safe alternative and marketed as “bladder friendly” [17], yet very little is known about the long term effects of MXE, and it may have the same effects on the bladder after long use as ketamine. Recently, a study conducted by Horsley et al. reported that MXE is more potent than ketamine [45]. Compared with ketamine, MXE is reported to have a delay in reaching its effect (90 min) with a longer duration time [46]. This might lead to overdose due to redosing if the user is expecting a faster ketamine onset [47]. A dose of MXE varies depending on the method of administration, between 20 to 100 mg for snorting, 10 to 80 mg for injection, and 40 to 100 mg orally [41]. Hospitalisations and deaths linked to accidental overdoses of MXE are reported [48, 49].

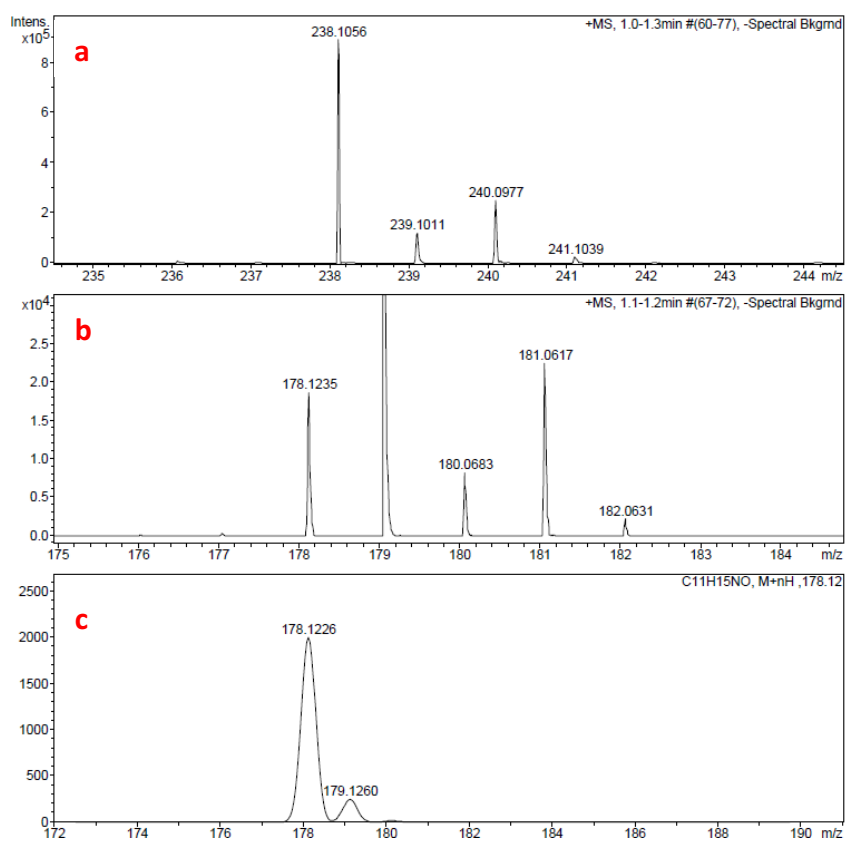
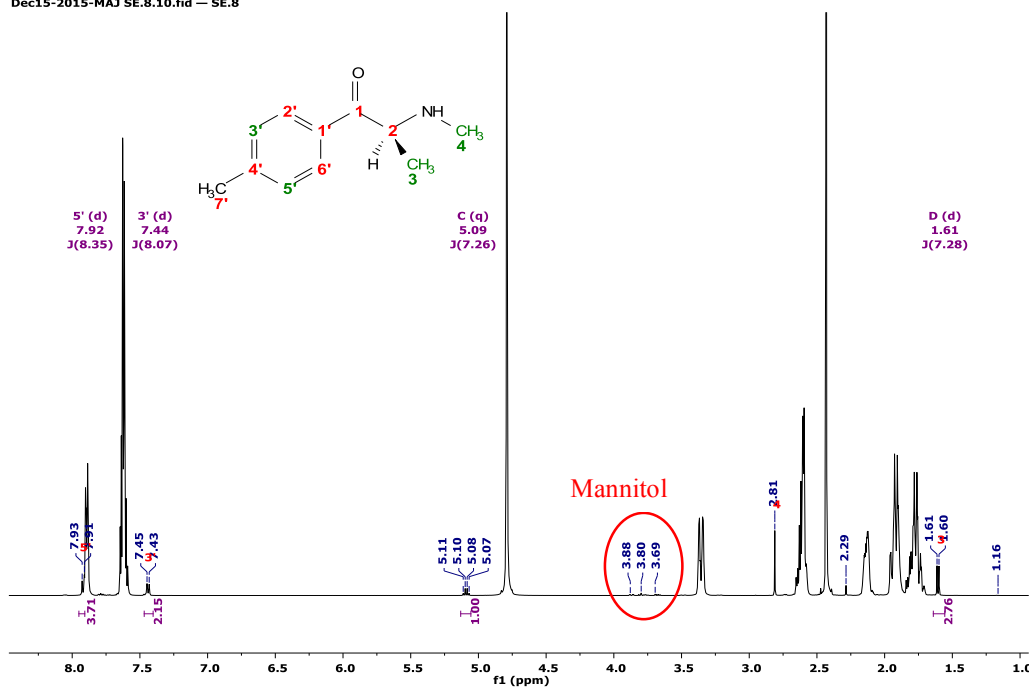


Fig. 22. Ketamine samples contained mephedrone and mannitol as cutting agents with their ¹H NMR assignments, and the MS spectrum confirmation of (a) ketamine, (b) mephedrone, and (c) simulated mass pattern of mephedrone

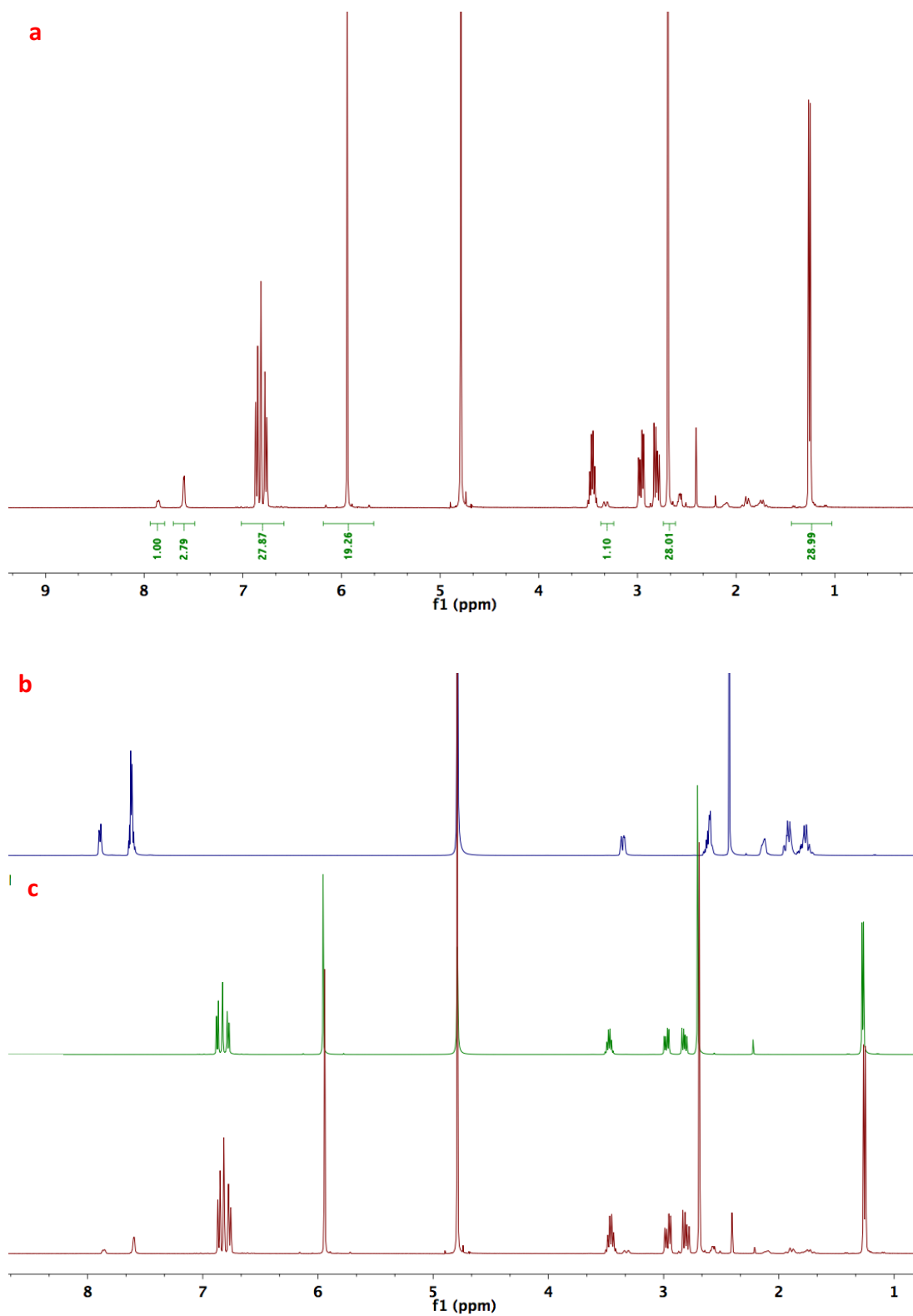


Fig. 23. ^1H NMR (D_2O) spectrum of (a) MDMA cut with ketamine in a ratio of 8.3:1 and a comparison to (b) authentic Ketamine, and (c) authentic MDMA

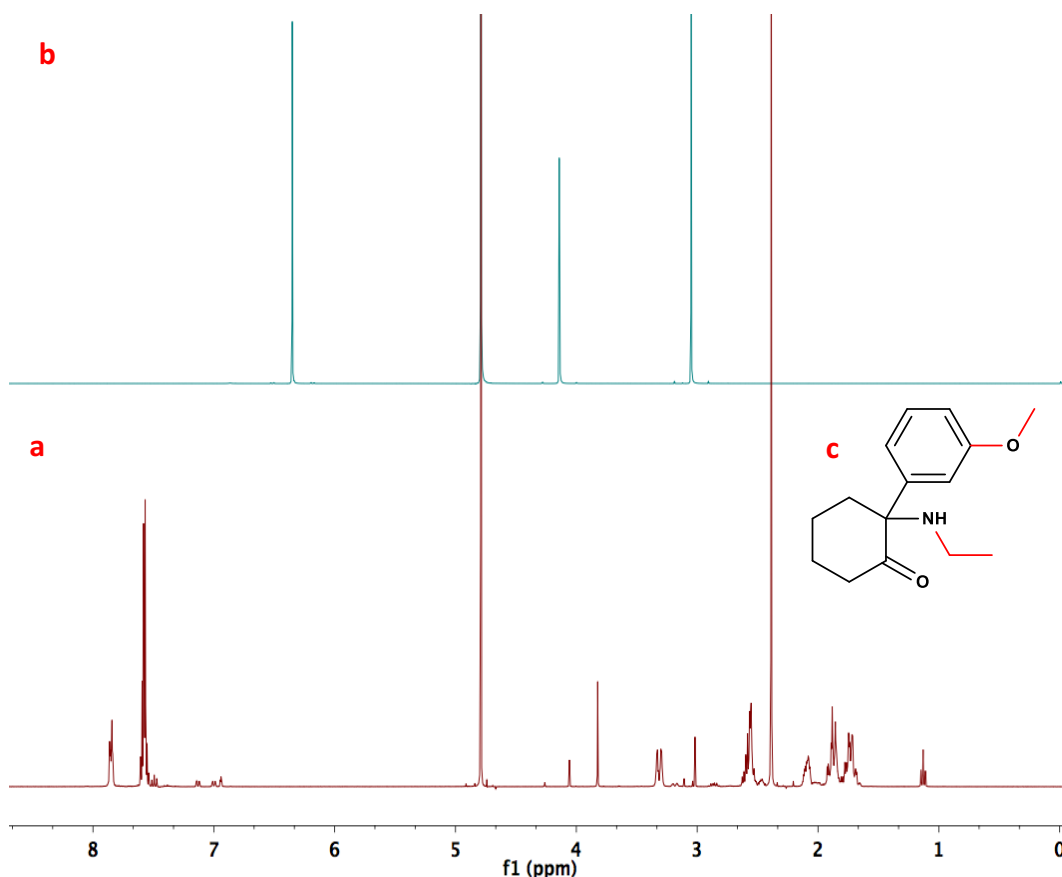


Fig. 24. (a) Ketamine sample cut with a combination of creatine and MXE, (b) authentic creatine spiked with maleic acid (6.20 ppm), and (c) MXE structure

Other possible impurities that can be present in the ketamine samples are synthetic precursors or solvents. These are introduced during ketamine production. Synthetic routes to ketamine are well documented in the literature [31, 32, 33]. Ketamine was synthesized via the following route: *o*-chloro benzonitrile (1) is used as starting material and reacted with a Grignard reagent (cyclopentylmagnesium bromide) to form *o*-chlorophenyl cyclopentyl ketone (2). This is then brominated to give (1-bromocyclopentyl)(2-chlorophenyl) methanone (3). The product is treated with liquid methylamine to form cyclopentanol product 4 and ketamine is achieved after heating to cause a ring-expansion (Fig. 25). Typical precursors involved in the production of illegal ketamine are cyclohexanone, methylamine, and chlorobenzene [34]. A method for the analogous MXE used 3-methoxy benzonitrile as the starting material followed by the same procedure as shown in Fig. 25 [38]. The use of a range of solvents, e.g. tetrahydrofuran (THF), benzene, is also described [28].

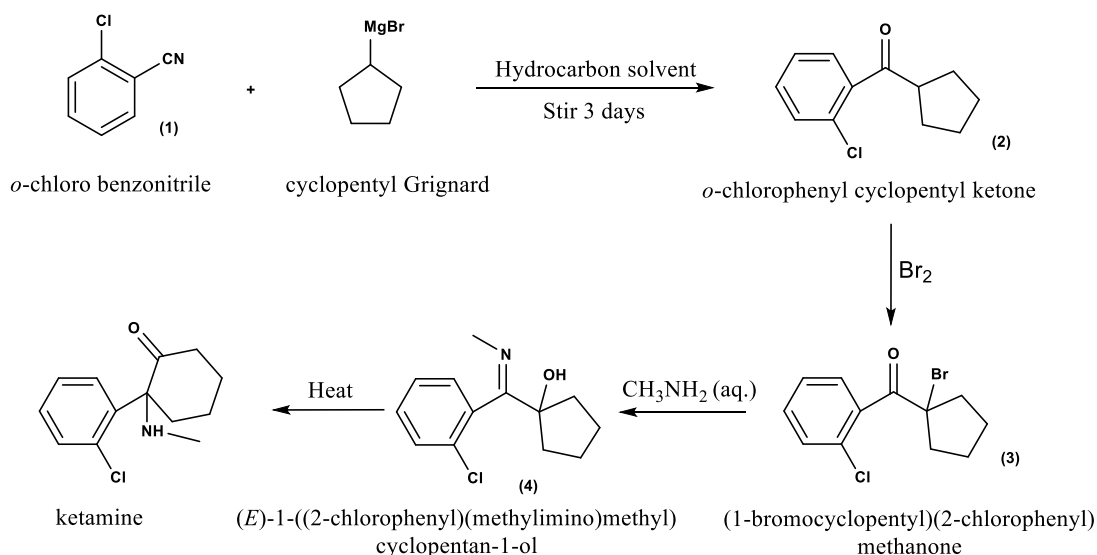


Fig. 25. Synthesis of ketamine

Trace impurities, either adulterants or solvents, are detected in the ^1H NMR of ketamine samples. These can be useful intelligence information as a characteristic markers to link between ketamine samples. Ketamine with the same impurity and with a same level might link to the same supplier. The most frequent solvent that was identified in the ketamine samples in this study was ethyl acetate, Fig. 26. Ethyl acetate is commonly used in extractions and its easy accessibility means it may be used during illicit manufacture of ketamine.

Traces of MDMA, paracetamol, mephedrone, and sucrose were identified (Figs. 27, 28, 29, and 30 respectively). 31 out of 64 street ketamine samples were found to have traces of MDMA. However, the MDMA was only present in very small amount, typically 1-2% of ketamine. Three different samples of the seized ketamine samples, two from Bristol, and one from Glastonbury amnesty bins were reanalysed three times each to confirm the MDMA contamination. One explanation for the presence of the MDMA is that it might be introduced during the ketamine handling as there are unlikely to be beneficial psychological affects to the abusers from these trace amounts. MDMA is more likely to be a contamination (impurity) rather than a cutting agent. However, it does raise the question about ketamine purity and whether (ab)users know what they are consuming.

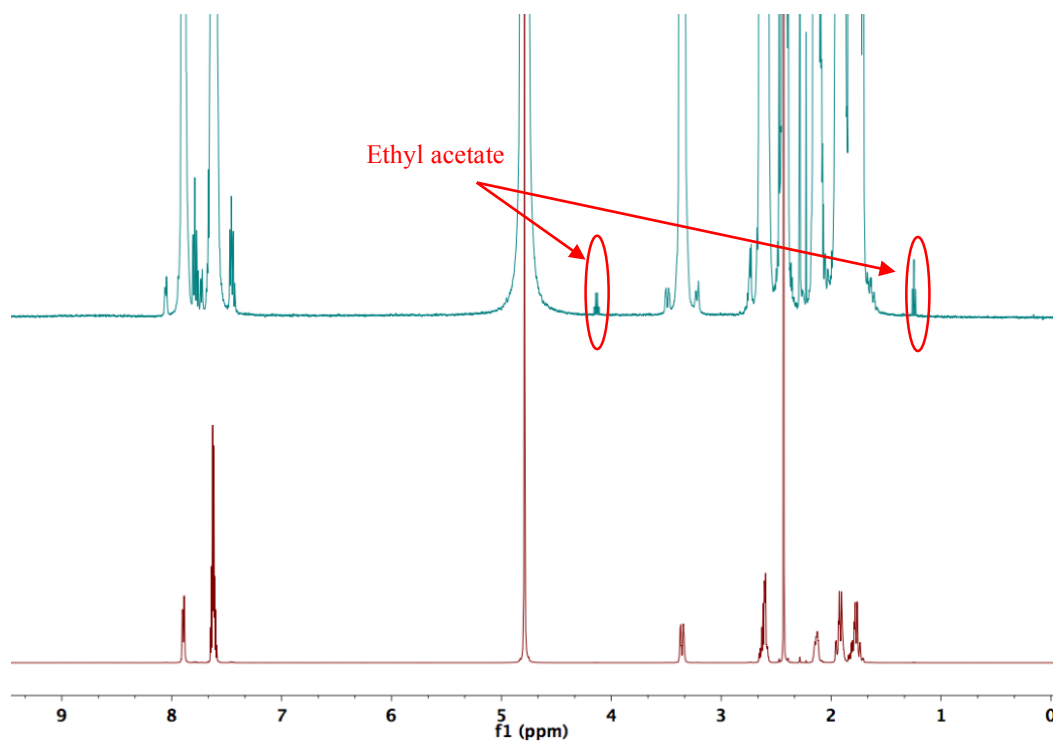


Fig. 26. ^1H NMR spectrum of a ketamine sample containing ethyl acetate solvent

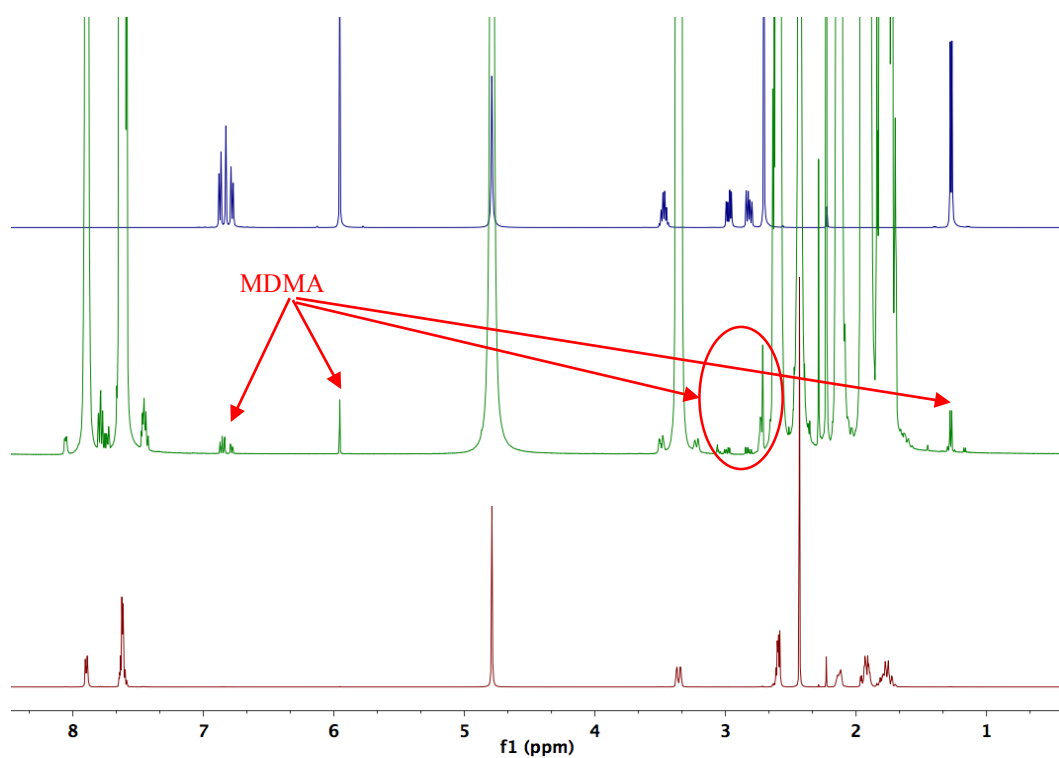


Fig. 27. Stacked spectra showing the MDMA contaminant level, $\geq 1\%$, in an illicit street ketamine sample

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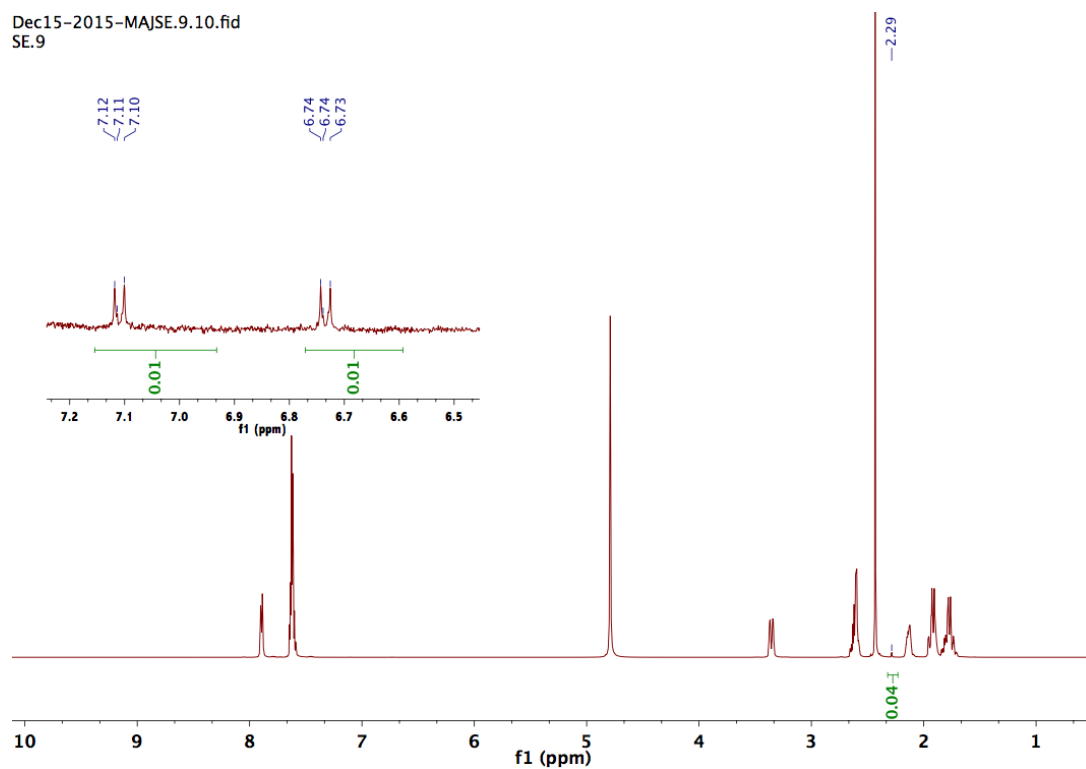


Fig. 28. Trace amounts of paracetamol in an illicit ketamine sample

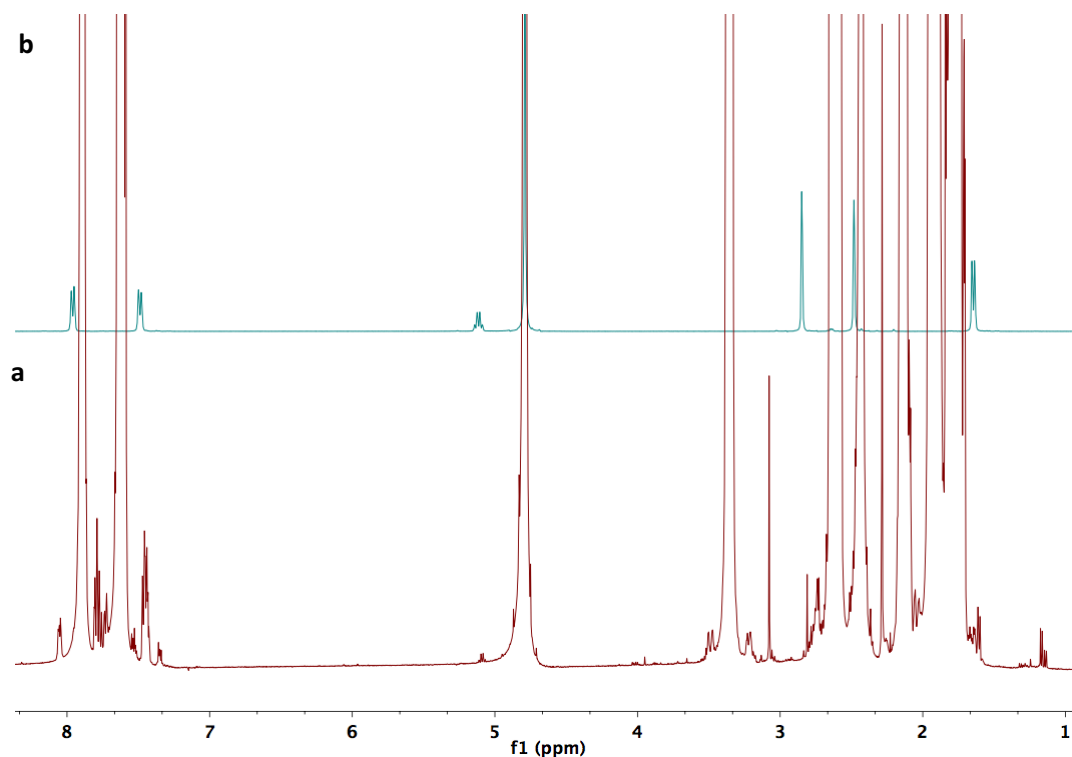


Fig. 29. ^1H NMR (D_2O) (a) expansion showing traces of mephedrone in an illicit ketamine sample compared to (b) an authentic mephedrone sample

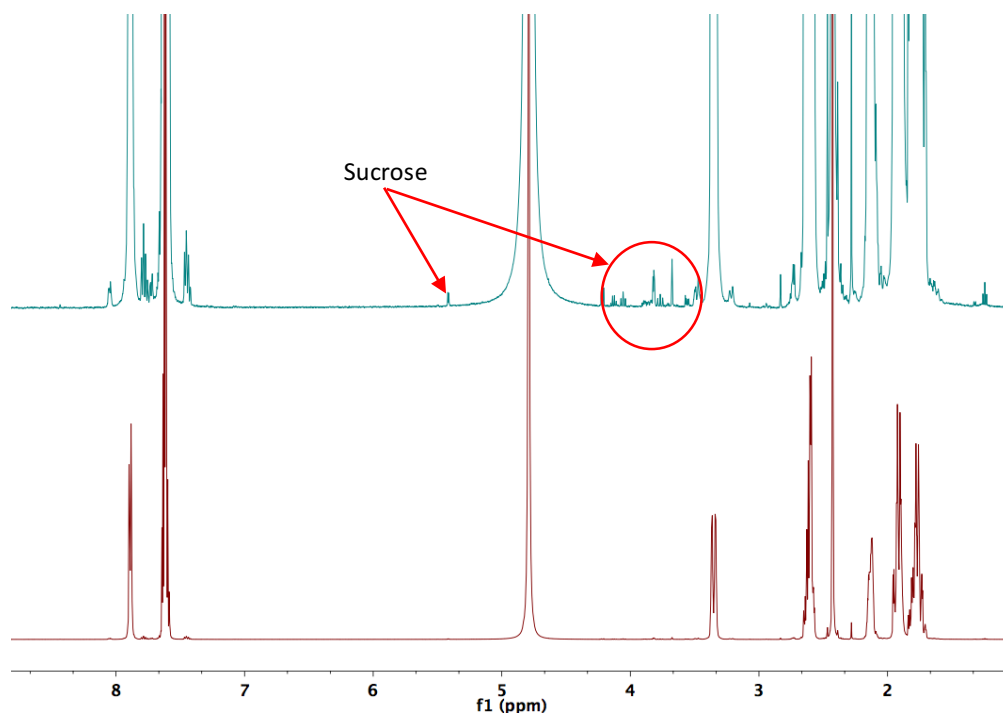


Fig. 30. Traces of sucrose in an illicit ketamine sample

6.3.4 Optical activity profiling of street and pharmaceutical ketamine samples

The ketamine samples were subjected to measurement of optical rotation in methanol (1 mg/mL). Equal amounts of both enantiomers are present in each of the seized, amnesty and pharmaceutical ketamine hydrochloride samples, $[\alpha]_D^{20} = 0$ ($c = 1$ mg/mL, MeOH). This suggested that the street ketamine samples are more likely to be synthesised illegally rather than diverted from legal overseas sources.

6.3.5 Isotope profiling of ketamine samples

6.3.5.1 EA-IRMS analysis

The EA-isotope ratio mass spectrometry (EA-IRMS) was employed as another method of profiling the ketamine samples and in particular their possible source. This technique is a bulk measurement technique and it is typically used to measure the average isotopic composition of organic compounds and to measure accurately variations in the abundance of isotopic ratios of light elements such as: $^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$, $^2\text{H}(\text{D})/^1\text{H}$, $^{15}\text{N}/^{14}\text{N}$, and $^{34}\text{S}/^{32}\text{S}$ [50]. Although, the isotopic

abundances of these elements were fixed when the Earth was formed, small variations can occur due to chemical, physical, and biological effects. These variations or fractions in the natural abundance of stable isotope are called δ , and these values are reported in parts per thousand or in parts per million (ppm) [51].

These analyses were applied to 14 samples of ketamine from different sources and used to determine the region of the world where the samples were produced (Table 2, *vide supra* 25 pp). The $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ values of the medical ketamine, VetalarTMV, KetalarTM, and an authentic ketamine HCl (manufactured in India) were compared with those samples seized from the street. Using EA-IRMS allows the analysis of ketamine samples without sample preparation avoiding any extraction step which can potentially add isotopic fractionation from the solvent. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ duplicate values of the 14 ketamine samples are plotted in Fig. 31.

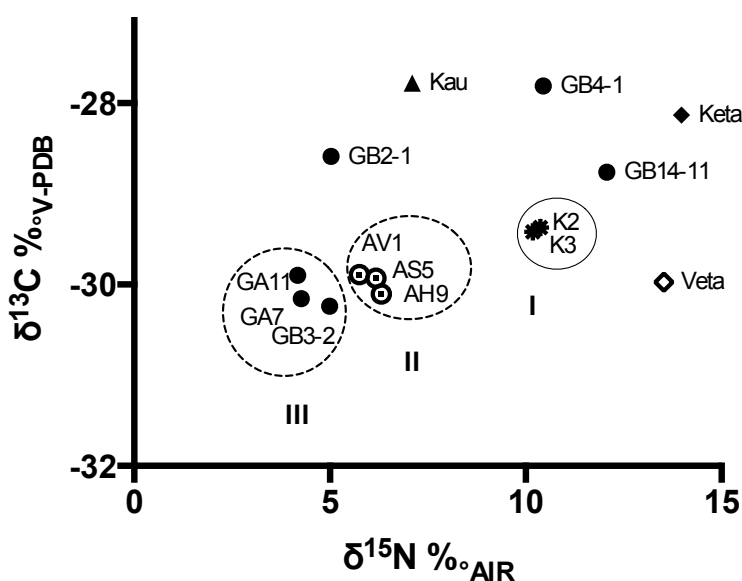


Fig. 31. $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ values for seized KT samples (*), Glastonbury amnesty bin (●), Bristol night club (◻), VetalarTMV (◇), KetalarTM (◆), and Ketamine HCl (▲), values are reported as the mean of duplicates

Based on the $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ (parts per thousand) values (Fig. 31), 8 of the samples could be grouped into three sets: I (seized samples), II (Bristol night club), and III (Glastonbury). The $\delta^{15}\text{N}$ ‰ isotopic ratios of the 11 samples varied between 4.175 and 13.975‰. These results showed that the seized ketamine samples (Group I) had almost identical values of $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ and therefore might be from a

single source. Similarly, all Bristol night club samples could be grouped together. The VetalarTMV and KetalarTM samples had different $\delta^{13}\text{C}\%$, but very close $\delta^{15}\text{N}\%$ values and did not match any of the illicit samples. None of the illicit samples had an isotopic ratio fingerprint comparable to the authentic ketamine (Indian). Three samples from the Glastonbury amnesty bin appeared to have distinctive profiles, GB2-1, GB14-11, and GB4-1.

6.3.5.2 Cluster analysis of $\delta^{13}\text{C}\%$, $\delta^{15}\text{N}\%$ and their %C and %N composition

The clustering test was applied on the mean values for the $\delta^{13}\text{C}\%$ and $\delta^{15}\text{N}\%$ beside their %C and %N. Gathering these four variables can provide more discrimination between the ketamine samples compared with using only the $\delta^{13}\text{C}\%$ and $\delta^{15}\text{N}\%$ values. The dendrogram (Fig. 32) revealed that the samples can each be grouped into two major clusters and two sub clusters.

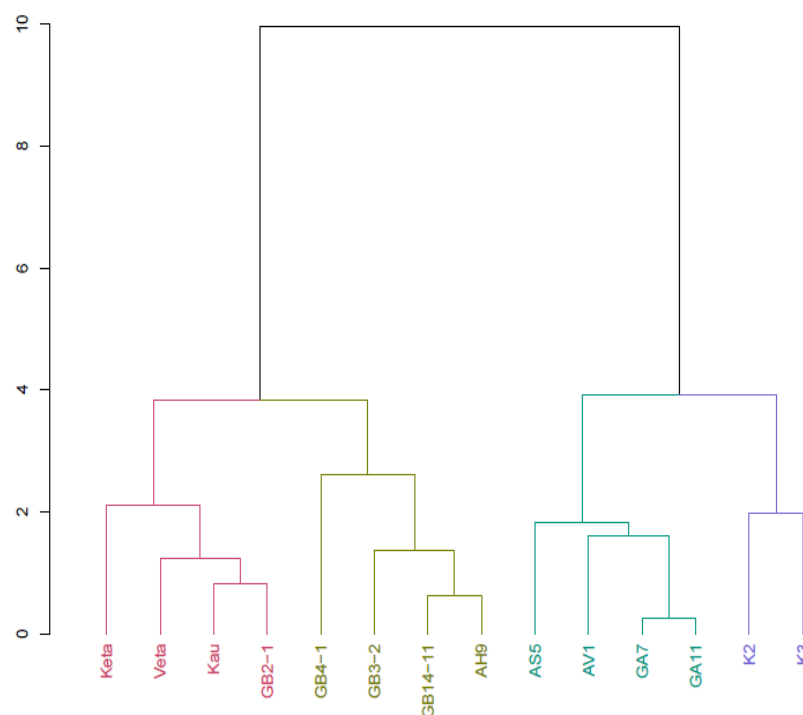


Fig. 32. Dendrogram on the $\delta^{13}\text{C}\%$ and $\delta^{15}\text{N}\%$ isotope values and their element percentages of street, medical (VetalarTMV and KetalarTM), seized and amnesty bins (Glastonbury and a Bristol night club) ketamine samples using average linkage between groups

In the first group, all the authentic and the medical ketamine are branched separately with only one sample (GB2-1) from the Glastonbury amnesty bin. GB2-1 has mean values very close to the authentic ketamine compared with VetalarTMV (◇) and KetalarTM. Therefore, GB2-1 might be manufactured in India and smuggled into the UK or the same starting materials might have been used to make these two.

In the second group, the two seized samples K2 and K3 are branched separately. By comparing with the scatter plot (Fig. 31), these two have very similar $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ and also close C and N % composition values. This suggests that K2 and K3 might come from the same source or same dealer.

For the reasons described above, EA-IRMS was used to predict ketamine samples sources and to detect any linkage between the samples. However, even though the sources of VetalarTMV, KetalarTM, and Indian ketamine are known, their precursor sources cannot be guaranteed. For example, if ketamine was manufactured in India with Chinese precursors, the ketamine will analyse as made in China.

6.3.6 X-ray single-crystal analysis

The stereochemical configurations (Fig. 5) of seized ketamine sample were analysed by a single-crystal X-ray diffraction (Fig. 33). The result shows the racemic mixture of the title compound. The crystal data of ketamine are given in Appendix 1.

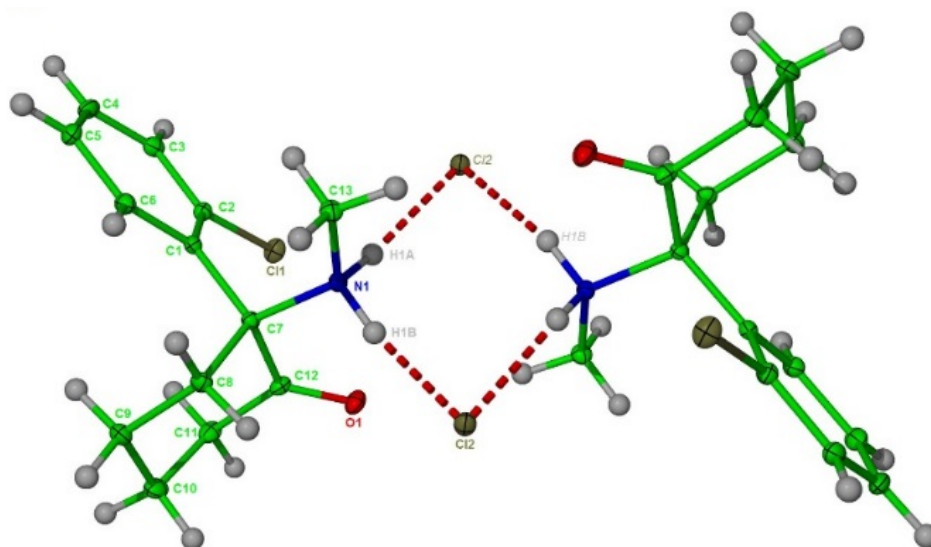


Fig. 33. X-ray structure of racemic ketamine

6.4. Conclusions

This study addresses the possibility that street ketamine may have been diverted from legal veterinary or human therapeutic sources in the UK. Medical ketamine in the UK is available in different formulations and under eight commercial trade names, all these contain excipients or preservatives, benzethonium chloride (BZC), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), or *p*-chlorocresol. ^1H NMR has been used to answer this question by profiling the impurity in the street samples of ketamine without any pre-step sample preparation. The spectroscopic data were compared with the legitimate ketamine samples, searching for any evidence of the presence of the listed preservatives. A ^1H NMR method has been developed and validated to detect the preservatives at concentrations as low as 30 $\mu\text{g/mL}$. Other impurities in ketamine samples, e.g. solvents, adulterants, were also investigated. The results indicate that none of the ketamine samples contain the preservatives used in the pharmaceutical products and therefore they are unlikely to have been diverted even though ketamine is not internationally controlled. Rather, these results support the growing evidence that street ketamine is more likely to be synthesised in clandestine laboratories abroad rather than diverted from legal sources.

Legal ketamine is available in the UK as a racemic mixture. The optical configurations of the illicit ketamine samples were investigated. That the mixtures were all racemic by polarimetry was also confirmed by an X-ray structure of an illicit (racemic) ketamine. There is potential that imported versions could be only *S*-ketamine, e.g. as used in Australia and the USA, which is about four times as potent as *R*-ketamine, and so with a concomitantly higher risk of overdose. Isotopic ratio analysis was also employed to investigate any possible link between legal ketamine, named VetalarTMV and KetalarTM and the street samples. None of the illicit samples had isotopic ratio $\delta^{13}\text{C}\text{‰}$ and $\delta^{15}\text{N}\text{‰}$ fingerprints equivalent (or even similar) to the VetalarTMV and KetalarTM samples, suggesting they had not been diverted from veterinary or hospital supplies. Furthermore, $\delta^{13}\text{C}\text{‰}$ can discriminate between VetalarTMV and KetalarTM compared to using only $\delta^{15}\text{N}\text{‰}$. With one exception (GB2-1), statistical analysis by dendrogram clustering successfully discriminated between the legal and illicit ketamine samples. This study shows the feasibility of using NMR in the impurity profiling of ketamine samples searching for possible preservatives in street samples.

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General conclusions

This research project has a focus on the impurity profiling of illicit drugs. In particular, on the NPS which constitute an emerging problem in the UK and elsewhere around the world. Chemical characterization of such NPS is challenging due to the high similarity of chemical structures between the samples and, added to that, the possible presence of other substances, e.g. adulterants. Among the main aims of this study is the development of a rapid, accurate, and practical analytical technique to analyse illicit drugs in street samples in order to be able to aid law enforcement. Different analytical techniques and methodologies were therefore tested throughout this study. In particular, the results show how the capabilities of NMR spectroscopy can be used in forensic drug analysis. One important aspect of this technique is the ability to provide accurate and quantitative chemical characterization of the drugs and their impurities even between highly similar drugs such as NPS and/or drugs in mixtures. From a practical point of view, using NMR spectroscopy as an additional analytical technique, complementary to other simpler methods, could be costly. High resolution NMR spectrometers usually have large and expensive superconducting magnets, e.g. a 500 MHz instrument may cost £500,000 before the £3,000 annual costs for essential coolants (liquid helium and nitrogen) and maintenance. However, good resolution can be achieved using less expensive, lower field-strength spectrometers, as found in research laboratories.

The first part of this investigation covered the analysis of amnesty bin contents as an important source of current drugs being abused. Two amnesty bins were analysed from a Bristol night club and from the Glastonbury music festival (2013). Findings from this kind of analysis provide useful information related to drugs' formulation, doses, adulterations, drugs' purity, and routes of administration. Illicit drugs emerged such as cathinones, e.g. flephedrone, mephedrone, 4-MEC, which were found to be impure, cut with benzocaine, creatine, sucrose, and MSG. This confirmed the viewpoint that drug purity is more likely to drop after biologically/pharmaceutically active compounds, e.g. "legal highs", have been banned so affecting their availability. Other not common but toxic drugs were identified in both amnesty bins, e.g. 2CB, 2CE, DMT. Both ketamine and mephedrone are still widely available and this might be because they are still not internationally controlled. Comparing the data from these two South-West region

amnesty bins, ketamine was the third most common drug in both. From this arises a concern about harm associated with the long-term abuse of ketamine with irreversible organ damage especially among the youngest abusers. Findings from this analysis can provide information among any newly abused drugs or/and medication, e.g. Modalert. These results were achieved by NMR spectroscopy, where a simple ^1H NMR experiment requires only minimal sample preparation and no need for reference materials, providing a quick (< 4 s, $NS = 16$), accurate, quantitative and non-destructive analytical technique.

On the national level, these data might contribute in mapping drugs from different regions in the UK. Internationally, a better picture can be provided about illicit drug trends by collaborating, sharing findings from amnesty bin analysis with other current information sources, e.g. waste water analysis, the internet based system, the Forensic Early Warning System (FEWS).

Recently, many other non-destructive methods have been introduced such as rapid screening methods to aid law enforcement, e.g. hand-held Raman, benchtop ATR-FTIR, and even benchtop NMR. An evaluation was carried out on a benchtop NMR system to test the ability of this instrument to detect drugs and their impurities in seized street samples. By experiment it was demonstrated that the benchtop NMR is unsuitable for the analysis of illicit drug and their impurities because of the poor spectrum resolution and the need for large amounts of sample (≈ 100 mg).

The close structural similarity between certain cathinone derivatives, e.g. mephedrone and flephedrone, can make the specific identification of these illicit drugs and their regioisomers challenging. This could lead to mislabelling with possible health and even legal consequences. Flephedrone, a cathinone derivative, was incorrectly assigned in its regioisomers in the literature. The combination of ^1H , ^{13}C , ^{15}N , and ^{19}F NMR spectroscopy was used to identify and decipher between flephedrone regioisomers and other cathinones such as mephedrone, showing the applicability of NMR for this kind of accurate analysis. Furthermore, 2D NMR spectroscopic techniques, ^{15}N HMBC and HOESY were applied in the analysis of flephedrone and mephedrone, allowing unambiguous assignment of the aromatic regions.

Flephedrone is being cut to increase street profits and this is the first evidence of cutting flephedrone with the topical anaesthetic benzocaine. Flephedrone and benzocaine have been separated with base-line resolution by GC-FID and the amount

of benzocaine adulterant quantified in the four (nightclub) flephedrone samples by GC; the adulterant levels determined by ^1H NMR integration values were in broad agreement. These data show that this now illegal cathinone-derived stimulant (a high) is now being cut by 5-12% with benzocaine, so drug users cannot be certain of the purity of the drug they are taking. Benzocaine as an adulterant can cause serious health problems, potentially more harmful than flephedrone. Therefore, there are risks from the pharmaceutically active cutting agents themselves. This is an urgent warning about benzocaine that needs to be heeded.

Drugs intelligence agencies in the analysis of illicit drugs are seeking two main important aspects. The first provides an accurate identity of the illicit drugs. The second investigates any relationships between the seized samples that can provide information on drugs networking and trafficking. Impurity profiling was used in this aspect to determine any similarity between mephedrone samples in chapter 5. 1D and 2D NMR provides unique and powerful analytical techniques for the impurity profiling of street mephedrone samples.

Molecules that can evade NMR spectroscopic detection are really few in number in comparison with other chromatographic and spectroscopic methods. Impurity profiling of mephedrone illustrated mephedrone samples were cut with safe, available and cheap materials, e.g. creatine, MSG, sucrose. The most common administration method of mephedrone is insufflation, with high and multiple doses, and harm associated with adulterants, e.g. benzocaine, MSG, and creatine, must be taken into account. Other recreational drugs, e.g. MDMA, are also used as adulterants for mephedrone in small amounts. However, pharmaceutical agents, e.g. benzocaine, a topical anaesthetic, is also a choice for an adulterant, perhaps selected to mimic the numbing effect of cocaine on the tongue/lips so that it might sold as cocaine. Moreover, benzocaine is shown to be used to cut other cathinones, e.g. flephedrone and this might link the mephedrone to another drug distributor.

Both mephedrone and their adulterants were quantified using quantitative NMR (qNMR). In order to quantify mephedrone another method, e.g. chromatography is really required. Hence, method development to be able to detect mephedrone and its adulterants, and the necessary authentic reference materials are required. Regarding the impurities (adulterants), 2D DOSY NMR was used to investigate the ability of this technique to separate the spectroscopic data in order to identify the cutting agents. The results showed promise in terms of impurity

separation with limitations linked to where the signals are overlapping. In reality, most of the mephedrone samples were cut with one or two adulterants. Consequently, this increases the chance to separate the impurities in the mephedrone samples by using DOSY.

To investigate the possible link between the street mephedrone samples, the ^1H NMR data were further examined by statistical analysis. The Principal Component Analysis (PCA), scree plot, was effective to classify between mephedrone samples with good agreement with the ^1H NMR impurity profiling analysis. The similarities and the differences between the samples for any possible link between them were linked by applying the dendrogram. This kind of analysis of mephedrone street samples with impurity profiling, quantification and statistical analysis can provide valuable information about availability and purity showing any possible link between the samples and maybe to the source of supply to aid law enforcement officers.

In the last chapter, one example where impurity profiling of an illicit drug can play an important role is addressed. From analysis of the amnesty bins' data, ketamine was the third drug of choice with 9.4% and 15.4% of the night club and of Glastonbury amnesty bins samples respectively. Ketamine is somewhat difficult to synthesise and some references suggest it might be diverted from hospitals, veterinary sources, or pharmaceutical companies. However, there is no literature evidence to prove the diversion of ketamine from legal sources. A comprehensive analysis was applied to ketamine samples investigating their impurities and possibly determining their origin.

Medical ketamine in the UK is available in different formulations and under seven commercial trade names, all have excipients. These excipients or preservative are BZC, EDTA, and CHLOR. ^1H NMR has been used to answer this question by profiling the impurity in the street samples of ketamine without any pre-step sample preparation. The data were compared with the medical ketamine searching for any evidence of the presence of any of the preservatives. A ^1H NMR method has been developed and validated to be able to detect the preservative as low as 30 $\mu\text{g/mL}$. Therefore, with high accuracy, other impurities such as solvents, synthetic precursors and adulterants were investigated. Evidence is presented of sucrose as an adulterant in ketamine samples.

The optical configuration of the illicit ketamine samples was investigated and

confirmed by an X-ray single crystal structure of the racemic ketamine. Legal Ketamine B.P. available in the UK is a racemic mixture of *S*- and *R*-isomers. There is the possibility that imported versions could be only *S*-ketamine, e.g. as used in Australia and the USA. This isomer is about four times as potent as *R*-ketamine, and therefore there is a concomitantly higher risk of overdose.

Isotopic ratio analysis was also employed to investigate the possible link between the legal ketamine, named VetalarTMV and KetalarTM, and the street samples. None of the illicit samples had isotopic ratio $\delta^{13}\text{C}\text{‰}$ and $\delta^{15}\text{N}\text{‰}$ fingerprints equivalent (or even similar) to the VetalarTMV and KetalarTM samples, suggesting they had not been diverted from veterinary or hospital supplies. Furthermore, $\delta^{13}\text{C}\text{‰}$ can discriminate between VetalarTMV and KetalarTM compared to using only $\delta^{15}\text{N}\text{‰}$. Statistical analysis by using dendrogram clustering could successfully discriminate between the legal and the other ketamine samples.

Our results indicate that none of the ketamine samples contain a preservative that is available in the pharmaceutical products and thus demonstrating that the possible diversion of ketamine from legal sources in the UK has not occurred. Ketamine is not internationally controlled, and this might suggest that ketamine might be diverted from another country into the UK. However, the results support the growing evidence that street ketamine is more likely to be synthesised rather than diverted from legal sources. This study shows the feasibility of NMR in the impurity profiling of ketamine samples searching for possible preservatives within the street ketamine samples. However, there has not been any previous NMR study reporting investigation of the possible diversion of ketamine from legal sources to the illicit drugs market.

Therefore, by achieving the six objectives set, the two major aims have been realised. The pharmaceutical analysis and impurity profiling of illicit drugs can contribute to a harm reduction approach. This is a complex problem that requires the sharing of knowledge from across widely interdisciplinary studies.

This analytical chemistry research project has deciphered different aspects across the wide range of impurities currently found in illicit drugs, with a focus on NPS and determining their purity/impurity (adulteration) by bringing together the use of many modern spectroscopic and chromatographic methods. A routine, rapid, and quantitative analytical protocol to identify illicit drugs and their impurities (cutting agents) in seized street samples has been developed.

Appendix 1. X-ray data of Ketamine

Table 1. Crystal data and structure refinement for ketamine.

Identification code	ketamine
Empirical formula	C ₁₃ H ₁₇ Cl ₂ N O
Formula weight	274.18
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2 ₁ /c
Unit cell dimensions	a = 7.0760(1) Å alpha = 90° b = 12.7980(2) Å beta = 103.465(1)° c = 15.2310(3) Å gamma = 90°
Volume	1341.38(4) Å ³
Z	4
Density (calculated)	1.358 Mg/m ³
Absorption coefficient	0.468 mm ⁻¹
F(000)	576
Crystal size	0.25 x 0.15 x 0.10 mm
Theta range for data collection	3.54 to 27.50°
Index ranges	-9 ≤ h ≤ 9; -16 ≤ k ≤ 16; -8 ≤ l ≤ 19
Reflections collected	5939
Independent reflections	5939 [R(int) = 0.0000]
Reflections observed (>2sigma)	5416
Data Completeness	0.997
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.912 and 0.823
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	5939 / 2 / 165
Goodness-of-fit on F ²	1.054
Final R indices [I>2sigma(I)]	R1 = 0.0368 wR2 = 0.1046
R indices (all data)	R1 = 0.0433 wR2 = 0.1086
Largest diff. peak and hole	0.292 and -0.323 eÅ ⁻³

Notes:

Equal amounts of both enantiomers are present in this crystal structure.

H1A and H1B are located and refined at a distance of 0.98 Å from N1.

Twinning (25%) about the 0,0,1 reciprocal axis vector was accounted for in this refinement.

The lattice is dominated by hydrogen bonded-dimers – each molecule-pair straddling a crystallographic inversion centre.

Table 2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1.U (eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

Atom	x	y	z	U(eq)
Cl(1)	-1023(1)	1513(1)	5434(1)	28(1)
Cl(2)	1574(1)	5719(1)	3816(1)	23(1)
O(1)	-727(2)	3117(1)	3802(1)	27(1)
N(1)	1714(2)	3898(1)	5182(1)	18(1)
C(1)	2797(2)	2102(1)	5681(1)	18(1)
C(2)	1429(2)	1496(1)	5984(1)	19(1)
C(3)	1944(3)	852(1)	6736(1)	24(1)
C(4)	3858(3)	782(1)	7197(1)	27(1)
C(5)	5258(2)	1383(1)	6934(1)	26(1)
C(6)	4724(2)	2035(1)	6187(1)	21(1)
C(7)	2306(2)	2846(1)	4874(1)	17(1)
C(8)	4013(2)	3007(1)	4407(1)	21(1)
C(9)	4474(2)	2005(1)	3950(1)	26(1)
C(10)	2724(3)	1626(1)	3239(1)	30(1)
C(11)	913(3)	1516(1)	3630(1)	27(1)
C(12)	606(2)	2518(1)	4085(1)	20(1)
C(13)	3157(2)	4431(1)	5916(1)	22(1)

Table 3. Bond lengths [\AA] and angles [$^\circ$] for ketamine.

Cl(1)-C(2)	1.7421(16)	O(1)-C(12)	1.214(2)
N(1)-C(13)	1.493(2)	N(1)-C(7)	1.5165(19)
C(1)-C(2)	1.400(2)	C(1)-C(6)	1.404(2)
C(1)-C(7)	1.530(2)	C(2)-C(3)	1.390(2)
C(3)-C(4)	1.375(3)	C(4)-C(5)	1.385(3)
C(5)-C(6)	1.391(2)	C(7)-C(12)	1.547(2)
C(7)-C(8)	1.551(2)	C(8)-C(9)	1.530(2)
C(9)-C(10)	1.523(3)	C(10)-C(11)	1.540(3)
C(11)-C(12)	1.498(2)		
C(13)-N(1)-C(7)	116.56(12)	C(2)-C(1)-C(6)	116.35(13)
C(2)-C(1)-C(7)	124.36(13)	C(6)-C(1)-C(7)	119.24(13)
C(3)-C(2)-C(1)	122.08(15)	C(3)-C(2)-Cl(1)	116.40(13)
C(1)-C(2)-Cl(1)	121.52(12)	C(4)-C(3)-C(2)	119.86(15)
C(3)-C(4)-C(5)	120.11(15)	C(4)-C(5)-C(6)	119.60(15)
C(5)-C(6)-C(1)	121.96(15)	N(1)-C(7)-C(1)	109.36(11)
N(1)-C(7)-C(12)	105.08(12)	C(1)-C(7)-C(12)	116.40(12)
N(1)-C(7)-C(8)	108.98(12)	C(1)-C(7)-C(8)	113.30(12)
C(12)-C(7)-C(8)	103.17(12)	C(9)-C(8)-C(7)	111.65(13)
C(10)-C(9)-C(8)	111.51(14)	C(9)-C(10)-C(11)	111.47(13)
C(12)-C(11)-C(10)	108.93(14)	O(1)-C(12)-C(11)	124.07(14)
O(1)-C(12)-C(7)	120.25(14)	C(11)-C(12)-C(7)	114.96(13)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1. The anisotropic displacement factor exponent takes the form: $-2 \pi^2 [h^2 a^{*2} U_{11} + \dots + 2 h k a^* b^* U_{12}]$.

Atom	U11	U22	U33	U23	U13	U12
Cl(1)	18(1)	29(1)	36(1)	5(1)	4(1)	-4(1)
Cl(2)	22(1)	22(1)	24(1)	3(1)	8(1)	1(1)
O(1)	25(1)	25(1)	28(1)	2(1)	-4(1)	1(1)
N(1)	18(1)	16(1)	19(1)	0(1)	4(1)	0(1)
C(1)	19(1)	17(1)	18(1)	-2(1)	5(1)	1(1)
C(2)	19(1)	18(1)	20(1)	0(1)	3(1)	-1(1)
C(3)	32(1)	18(1)	23(1)	2(1)	10(1)	-1(1)
C(4)	38(1)	21(1)	18(1)	4(1)	2(1)	7(1)
C(5)	24(1)	30(1)	22(1)	1(1)	0(1)	7(1)
C(6)	19(1)	24(1)	21(1)	-1(1)	4(1)	0(1)
C(7)	18(1)	16(1)	16(1)	-1(1)	4(1)	0(1)
C(8)	21(1)	24(1)	19(1)	2(1)	8(1)	0(1)
C(9)	29(1)	27(1)	24(1)	0(1)	10(1)	5(1)
C(10)	43(1)	25(1)	21(1)	-4(1)	8(1)	4(1)
C(11)	32(1)	21(1)	23(1)	-3(1)	-2(1)	-2(1)
C(12)	21(1)	20(1)	18(1)	3(1)	0(1)	-3(1)
C(13)	21(1)	22(1)	22(1)	-4(1)	2(1)	-2(1)

Table 5. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for ketamine.

Atom	x	y	z	U(eq)
H(3)	977	460	6931	29
H(4)	4219	322	7698	32
H(5)	6574	1349	7262	32
H(6)	5693	2447	6013	26
H(8A)	3672	3568	3950	25
H(8B)	5181	3232	4862	25
H(9A)	5571	2136	3663	31
H(9B)	4878	1454	4412	31
H(10A)	3029	941	3003	36
H(10B)	2447	2127	2730	36
H(11A)	-240	1359	3139	32
H(11B)	1097	934	4070	32
H(13A)	3453	3983	6453	33
H(13B)	2616	5095	6065	33
H(13C)	4351	4566	5713	33
H(1A)	506(16)	3856(15)	5386(12)	24(5)
H(1B)	1530(30)	4380(12)	4670(9)	30(5)

Table 6. Dihedral angles [°] for ketamine.

Atom1 - Atom2 - Atom3 - Atom4	Dihedral
C(6) - C(1) - C(2) - C(3)	-0.8(2)
C(7) - C(1) - C(2) - C(3)	-178.28(14)
C(6) - C(1) - C(2) - Cl(1)	179.10(11)
C(7) - C(1) - C(2) - Cl(1)	1.6(2)
C(1) - C(2) - C(3) - C(4)	-1.2(2)
Cl(1) - C(2) - C(3) - C(4)	178.92(13)
C(2) - C(3) - C(4) - C(5)	2.4(2)
C(3) - C(4) - C(5) - C(6)	-1.7(3)
C(4) - C(5) - C(6) - C(1)	-0.4(2)
C(2) - C(1) - C(6) - C(5)	1.6(2)
C(7) - C(1) - C(6) - C(5)	179.20(14)
C(13) - N(1) - C(7) - C(1)	57.48(16)
C(13) - N(1) - C(7) - C(12)	-176.87(12)
C(13) - N(1) - C(7) - C(8)	-66.86(16)
C(2) - C(1) - C(7) - N(1)	85.63(17)
C(6) - C(1) - C(7) - N(1)	-91.82(16)
C(2) - C(1) - C(7) - C(12)	-33.2(2)
C(6) - C(1) - C(7) - C(12)	149.34(14)
C(2) - C(1) - C(7) - C(8)	-152.60(15)
C(6) - C(1) - C(7) - C(8)	29.96(18)
N(1) - C(7) - C(8) - C(9)	-171.10(12)
C(1) - C(7) - C(8) - C(9)	66.91(16)
C(12) - C(7) - C(8) - C(9)	-59.81(15)
C(7) - C(8) - C(9) - C(10)	59.25(18)
C(8) - C(9) - C(10) - C(11)	-53.22(19)
C(9) - C(10) - C(11) - C(12)	52.16(18)
C(10) - C(11) - C(12) - O(1)	110.20(18)
C(10) - C(11) - C(12) - C(7)	-60.03(17)
N(1) - C(7) - C(12) - O(1)	6.01(19)
C(1) - C(7) - C(12) - O(1)	127.15(15)
C(8) - C(7) - C(12) - O(1)	-108.13(17)
N(1) - C(7) - C(12) - C(11)	176.65(13)
C(1) - C(7) - C(12) - C(11)	-62.21(18)
C(8) - C(7) - C(12) - C(11)	62.51(16)

Symmetry transformations used to generate equivalent atoms: ...

Appendix 2. Presentations arising from these studies

Refereed Publication:

^1H , ^{13}C , ^{15}N HMBC, and ^{19}F NMR spectroscopic characterisation of seized flephedrone, cut with benzocaine. J Pharm Biomed Anal. 2015 Mar 25; 107:535-8.
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Oral Presentations:

1. “Analysis of a Bristol amnesty bin as an indicator of current drugs trends” APS PharmSci, 8th Sep 2014, University of Hertfordshire, UK.
2. “What is being used and from where is it coming? Analysis of amnesty bin contents” Symposium for PhD students Researching Substance (Mis)use (SSA), 10th July 2015, University of Bath, UK.
3. “To determine the geographical origin of street ketamine samples by using ^{13}C and ^{15}N stable isotope ratio analysis” 4th International Conference on Forensic Research & Technology, 29th Sep 2015, Atlanta, USA.
4. “Forensic analysis of a Glastonbury amnesty bin: Evaluation of compact spectrometer techniques compared to NMR” 5th International Conference on Forensic Research & Technology, Oct 31st-Nov 2nd, 2016 San Francisco, California, USA.

Poster Presentations:

1. “Profiling seized samples of mephedrone adulterated with creatine” APS PharmSci, 2nd-4th Sep 2013, Heriot-Watt University, Edinburgh UK.
2. “Fast analytical methods to profile three seized ketamine samples” 2nd International Conference on Novel Psychoactive Substances, 12th-13th Sep 2013, Swansea, UK.
3. “Analysis of a Bristol amnesty bin as an indicator of current drugs trends” APS PharmSci, 8th-10th Sep 2014, Univ of Hertfordshire, Hertfordshire, UK.
4. “Chemical characterisation of seized flephedrone by ^1H , ^{13}C , and ^{19}F NMR spectroscopy” 3rd International Conference on Novel Psychoactive Substances, 15th-16th May 2014, Rome, Italy.

5. “Carbon and nitrogen stable isotope ratio analysis of seized and amnesty bin ketamine samples to determine their geographical origin” APS PharmSci, 8th Sep 2015, Nottingham, UK.
6. “Impurity profiling of seized and amnesty bin ketamine samples to determine if any diversion from hospital or veterinary sources to drug users in the UK” APS PharmSci, 8th Sep 2015, Nottingham, UK.
7. “Chemical profiling of MDMA samples and their impurities within a clinical study on psychoactive effects of MDMA on users” APS PharmSci, 8th Sep 2015, Nottingham, UK.
8. “Impurity profiling of MDMA tablets in the UK: A combination of benchtop FT-IR equipped with TICTAC database and ^1H NMR” APS PharmSci, 7th Sep 2016, Glasgow, UK.



Short communication

 ^1H , ^{13}C , ^{15}N HMBC, and ^{19}F NMR spectroscopic characterisation of seized flephedrone, cut with benzocaine

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ABSTRACT

Flephedrone (4-fluoromethcathinone, 4-FMC) was analysed using ^1H , ^{13}C , ^{15}N HMBC, and ^{19}F observe spectroscopy, gas chromatography-flame ionisation detection (GC-FID), and electrospray ionisation-mass spectrometry (ESI-MS). Analysis of four 4-FMC samples (from a Bristol nightclub in 2013) showed that they all contained benzocaine as the cutting agent present in different amounts from 5 to 12%. Using these methods, we successfully differentiated between flephedrone regioisomers and mephedrone in an analytical method validated for flephedrone as a substituted cathinone. The data show that these now illegal cathinone-derived stimulants (highs) are now being cut; users cannot be certain of the purity of the drug they are taking. Furthermore, there are risks from the pharmaceutically active cutting agents themselves.

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1. Introduction

The close similarity between cathinone derivatives (Fig. 1) makes the specific identification of these drugs and their regioisomers challenging and could lead to mislabelling or accidental misidentification or deliberate deception as in the nature of the cutting of illicit (street) drugs. Flephedrone (4-fluoromethcathinone, 4-FMC) (Fig. 1c) was first synthesised in 1952, but surprisingly there is a paucity of published data for this compound. Compared with cathinone (Fig. 1a) and mephedrone (4-methylmethcathinone, 4-MMC) (Fig. 1b), the C–F bond on the aromatic ring of 4-FMC (Fig. 1c) has a specific polarity effect. This may influence receptor binding and therefore influence the biological activity [1,2]. The biological effects of cathinone ring-substituted derivatives are claimed to be similar to cocaine, amphetamine, or MDMA (ecstasy) [3]. In the UK, a question about a decrease in the purity of cathinones has arisen after they were banned as Class B drugs under the Misuse of Drugs Act in April 2010 [4].

As a routine analytical technique, e.g. used by the police, GC–MS [5–7] or GC-FID [6], infrared (FT-IR) [7], and Raman spectroscopy are widely used for the detection of controlled substances [8]. Nevertheless, standard reference materials for detecting what may be present in unknown samples are required.

Using GC–MS alone would potentially not distinguish between the 2-, 3-, and 4-regioisomers of FMC, all the isomers having been reported in elegant synthetic work with full characterisation by Archer [7]. Electron impact-mass spectrometry (EI-MS) also leads to uncertainty in distinguishing between flephedrone isomers [7]. More recently, screening methods for the detection of new (illicit) psychoactive drugs in urine were reported that use liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) [9], HPLC [6], and ultra performance liquid chromatography–quadrupole time-of-flight-mass spectrometry (UPLC–QTOF-MS) [5].

NMR spectroscopy is an established analytical tool for the identification of controlled substances without the need for sample derivatisation or the need to remove any impurity. Also, distinguishing between regioisomers, between salt and free base, determining percentage purity and impurity ratios are successfully and rapidly achieved using NMR spectroscopy. Police or forensic intelligence information may be gained from this kind of analysis to aid law enforcement. The aim of this work is to develop a robust, rapid, and quantitative analytical approach to detect flephedrone as well as the impurities (cutting agents) present in street samples using ^1H , ^{13}C , ^{15}N , and ^{19}F NMR spectroscopy together with GC.

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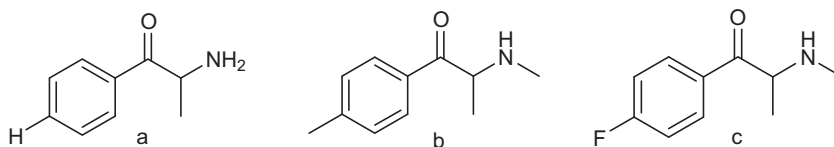


Fig. 1. (a) Cathinone, (b) mephedrone, and (c) flephedrone.

2. Experimental

2.1. Chemicals and materials

Four creamy coloured crystal samples, as part of an amnesty bin provided in a (Bristol, UK) nightclub, in 2013, were identified as flephedrone. A reference standard of flephedrone hydrochloride salt (4-FMC HCl) was purchased from LGC Standards, UK. An analytical standard of mephedrone hydrochloride salt (4-MMC HCl) was purchased from Aldrich, UK. Deuterium oxide (99.8 atom % D, Aldrich, UK), per-deuterated methanol (99.8 atom % D, Cambridge Isotope Laboratories, USA), and all other solvents were of HPLC grade, $\geq 99.9\%$ purity (Aldrich, UK). Benzocaine B.P. was purchased from J. M. Loveridge plc (Southampton, UK).

2.2. Experimental procedures and instrumentation

Each sample of flephedrone (4-FMC) was weighed, dissolved, and analysed at 20–25 °C without any purification. NMR spectra were recorded on Bruker 400 or 500 MHz spectrometers, referenced to external Me_4Si and/or via residual protons in the deuterated solvents. Samples (~ 30 mg each) were vortexed for 2 min in D_2O (0.6 mL). Identification of flephedrone samples and their impurities followed NMR analysis including: ^1H , ^{13}C , ^{15}N (HMBC), HSQC, HMBC, and COSY. A GC method was optimised to analyse authentic 4-FMC nightclub samples dissolved in methanol (50 mg/mL), and benzocaine B.P. injected directly into the GC-FID without any prior chemical derivatisation. GC separation was performed on a CP-9003 (Chrompack, Middelburg, The Netherlands) using a capillary column ZB-WAX (polyethylene glycol, 10 m \times 0.25 mm \times 0.25 μm , Phenomenex, UK). An injection volume (1–2 μL) was carried by helium carrier gas at a flow rate of 23–27 mL/s. The oven temperature was programmed with 250 °C injector and detector temperature, 110 °C (2 min) oven initial and 230 °C (4 min) final temperatures with 40 °C/min rate of heating, total run time was 9 min. Positive ion $[\text{M}+\text{H}]^+$ mode LC/MS was performed, on samples dissolved in methanol, using a Bruker Daltonics micrOTOF mass spectrometer equipped with an electrospray ionisation (ESI) source.

3. Results and discussion

3.1. ^1H , ^{13}C , ^{15}N , and ^{19}F NMR spectroscopy

4-FMC NMR spectra show diagnostic ^{19}F and ^1H coupling (Fig. 2) in the aromatic region; ^1H , ^{13}C , and ^{19}F NMR spectral data are reported in Table 1. NMR spectra are discussed and compared to those of the closely related cathinone derivative mephedrone 4-MMC (Table 1, Fig. 2). The structures were confirmed by comparing the NMR data with those of an authentic sample of 4-FMC and the literature values [7], establishing the regioisomer as *para*-substituted. Unfortunately, two lines in the literature ^{13}C NMR spectroscopic assignment Table have been transposed [7], and the correct aromatic region assignment is given in Table 1 for δ 116 (d, $^2J_{\text{CF}}$ 22 Hz) and 132 (d, $^3J_{\text{CF}}$ 10 Hz) ppm, where the coupling constants are diagnostic for *ortho* to F (C3, C5) and *meta* to F (C2, C6) respectively.

While the ^1H NMR spectrum of 4-FMC is similar to that of 4-MMC (Fig. 2), the absence of a 4-methyl aromatic substituent peak, and the coupling from the ^{19}F atom are diagnostic. The ^{13}C - ^{19}F splitting patterns around the entire aromatic ring (e.g. 167 ppm, d, $^1J_{\text{CF}}$ 255 Hz; 129 ppm, d, $^4J_{\text{CF}}$ 3 Hz) in the ^{13}C spectra (Table 1) are also significant. ^{15}N NMR HMBC was run to detect the number, kind, and position of the nitrogen atoms proving the presence of a secondary amine coupled to the two *N*- and α -methyl groups (Fig. 3). ^{19}F NMR spectroscopy proves the presence and position of a fluorine atom in such samples. Comparing with the literature data [7], the ^{19}F NMR chemical shift values for the each of the four 4-FMC samples show *para*-substitution: ^{19}F NMR showed -102.1 ppm (tt, $^3J_{\text{HF}}$ 8.4 Hz, $^4J_{\text{HF}}$ 5.2 Hz) (Fig. 3) with the d $^1J_{\text{CF}}$ 255 Hz easily visible only in the ^{13}C NMR spectrum, not in the ^{19}F NMR spectrum due to the low (1.1%) ^{13}C natural abundance.

3.2. ESI-MS and impurity profiling

The ESI-MS gave $[\text{M}+\text{H}]^+$ at 182.0976 and 182.1006 for the samples and the authentic hydrochloride salt of flephedrone respectively, $\text{C}_{10}\text{H}_{13}\text{FNO}$ requires 182.0981 thus confirming the identity of a regioisomer of flephedrone. ESI-MS spectra also gave $[\text{M}+\text{H}]^+$ 166.0874. Benzocaine $\text{C}_9\text{H}_{12}\text{NO}_2$ $[\text{M}+\text{H}]^+$ requires 166.0868; $[\text{M}+\text{Na}]^+$ $\text{C}_9\text{H}_{11}\text{NO}_2\text{Na}$ found 188.0689, requires 188.0687.

Analysis of the 4-FMC samples by NMR and ESI-MS showed that they were impure. The ^1H and ^{13}C NMR data of the impurity are: ^1H NMR (D_2O): δ 1.36 (t, $^3J_{\text{HH}}$ 7 Hz), 4.33 (q, $^3J_{\text{HH}}$ 7 Hz), 6.83 (H3 and H5, t, $^3J_{\text{HH}}$ 7.6 Hz), 7.86 (H2 and H6, d, $^3J_{\text{HH}}$ 7.6 Hz) [10]; ^{13}C NMR (D_2O): δ 15 (C7), 61 (C6), 114 (C3, C5), 119 (C1), 132 (C2, C6), 155 (C4), 169 (C5) ppm [11]. A GC-FID method was then developed to resolve flephedrone and benzocaine in mixed samples, achieved with retention times of $R_t = 2.8$ min and 4.9 min respectively (Fig. 4). The benzocaine levels in these 4-FMC samples were quantified by GC (5, 6, 7, and 12%), impurity levels which are in agreement with those determined from the (more approximate) ^1H NMR integration data, 4, 6, 7, and 10%. Benzocaine has previously also been quantified by HPLC [12].

4-FMC is a designed cathinone derivative either designed to side-step the banning of the legal high 4-MMC and/or with one eye on the potential pharmacological enhancement from incorporating a fluorine substituent in a biologically active small molecule as “there is also evidence that single, carbon-bound fluorine substituents, particularly when on an aromatic ring, can exhibit specific polarity influences, including H-bonding, that can strongly influence binding” [1]. The human toxicology of flephedrone is not well established [13]. Lipophilicity is significant as it often controls the absorption, transport, or receptor binding of a compound and so can enhance its bioavailability. The effect on the lipophilicity of fluorine substituents on an aromatic ring was calculated by Dolbier showing that CF_3 ($\pi_x = 0.88$) is greater than CH_3 ($\pi_x = 0.56$) following from the respective *n*-octanol/water partition coefficients [1]. So, perhaps counter-intuitively, the presence of the most powerful electronegative atom, a fluorine substituent, gives rise to enhanced lipophilicity.

Benzocaine is a well-known topical anaesthetic. However, the FDA has reported that using a gel or spray containing

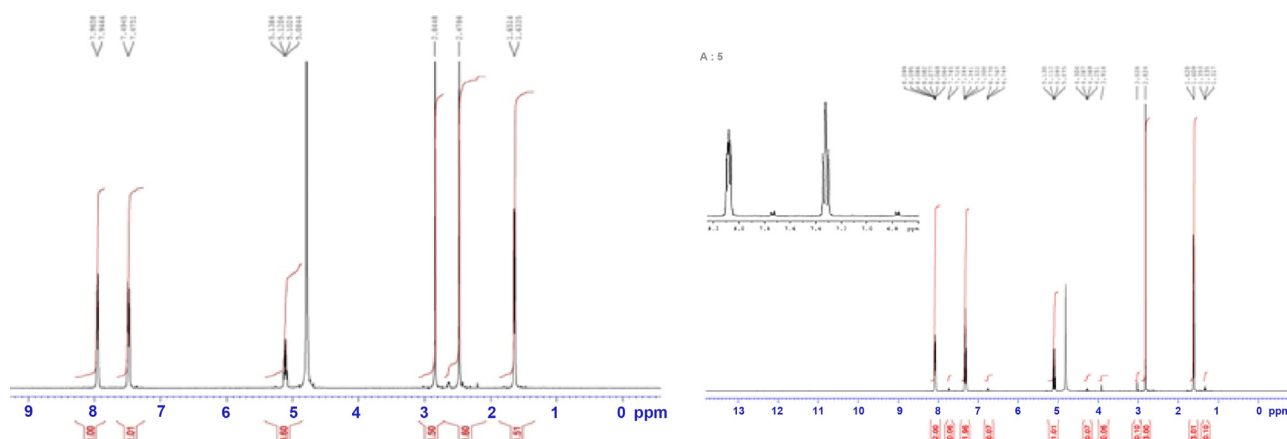


Fig. 2. ^1H NMR of mephedrone (left) and flephedrone (right) (D_2O) with aromatic region expansion showing ^{19}F couplings and the $\sim 7\%$ benzocaine signals here are clearly visible.

Table 1

^1H (400 MHz), ^{13}C (100 MHz), and ^{19}F (470 MHz) spectral data of flephedrone (D_2O).

Position	Flephedrone			Mephedrone	
	^1H	^{13}C	^{19}F	^1H	^{13}C
1	–	129 (d, $^4J_{\text{CF}}$ 3 Hz)	–102.1, tt, 3J 8.4, 4J 5.2 Hz	–	129.7
2,6	8.07 (dd, $^3J_{\text{HH}}$ 8.4 Hz, $^4J_{\text{HF}}$ 5.2 Hz)	132 (d, $^3J_{\text{CF}}$ 10 Hz)		7.96 (d, $^3J_{\text{HH}}$ 8 Hz)	129.1
3,5	7.31 (t, $^3J_{\text{HH}} = ^3J_{\text{HF}}$ 8.4 Hz)	116 (d, $^2J_{\text{CF}}$ 22 Hz)		7.48 (d, $^3J_{\text{HH}}$ 8 Hz)	129.9
4	–	167 (d, $^1J_{\text{CF}}$ 255 Hz)		–	147
7	–	196		–	197
8	5.09 (q, $^3J_{\text{HH}}$ 7 Hz)	60		5.11 (q, $^3J_{\text{HH}}$ 7 Hz)	60
9	1.60 (d, $^3J_{\text{HH}}$ 7 Hz)	15		1.64 (d, $^3J_{\text{HH}}$ 7 Hz)	15
N-CH ₃	2.81, s	31		2.84, s	31
4-Me	–	–		2.48, s	21

benzocaine could cause a serious blood disorder, methemoglobinemia [14,15]. Similar concerns are raised elsewhere, e.g. Taleb and co-workers review the evidence where even following topical application of benzocaine, oxidised haemoglobin might increase causing methemoglobinemia [16]. Even more recently, a few weeks ago, a case of acquired methemoglobinemia due to the frequent use of the legal high “Pink Panthers” was reported in parallel with the increasing prevalence of legal highs particularly those containing added benzocaine [17]. Benzocaine is currently being used in the illicit drug market as a common adulterant (cutting agent) to increase profit [18], indeed it is now commonly found in cocaine hydrochloride [18–20], which might link the supply of such adulterated flephedrone to that of other commonly cut illicit drugs.

Drug dealers may use benzocaine as it is easily accessible and similar in physical appearance to flephedrone. In sufficient quantity, benzocaine might be added to flephedrone in order to mimic the initial numbing (anaesthetic) effect of cocaine [21] and possibly even to sell the mixture not as flephedrone but as cocaine.

Our rapid analysis and quantification of 4-FMC and benzocaine compare well with the literature methods [7,12]. Research is also on-going to replace aspects of wet-chemistry based quantitative analysis on minimal trace amounts of illegal drugs with rapid in situ (e.g. potentially on-site analysis in a nightclub) screening using handheld (therefore portable) spectrophotometers [22,23]. The use of Raman spectroscopy to detect counterfeit medicines [22] may be extended to the analysis of street drugs [23], whilst

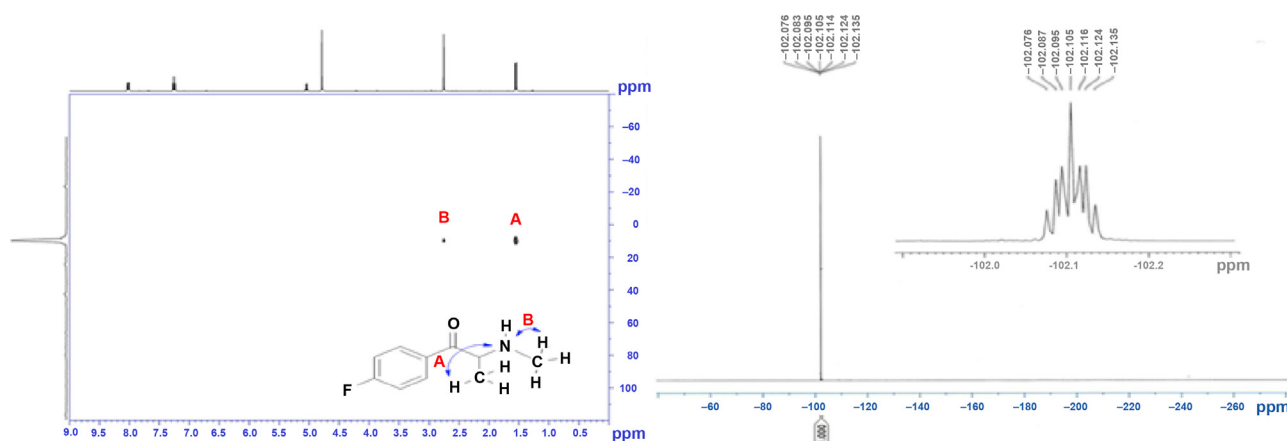


Fig. 3. ^{15}N -HMBC (500 MHz) spectrum of flephedrone (D_2O) (left) showing two ^{15}N – ^1H couplings (with peak assignments) and ^{19}F NMR spectrum (^1H coupled) of flephedrone (D_2O) (right) (in the presence of $\sim 7\%$ benzocaine).

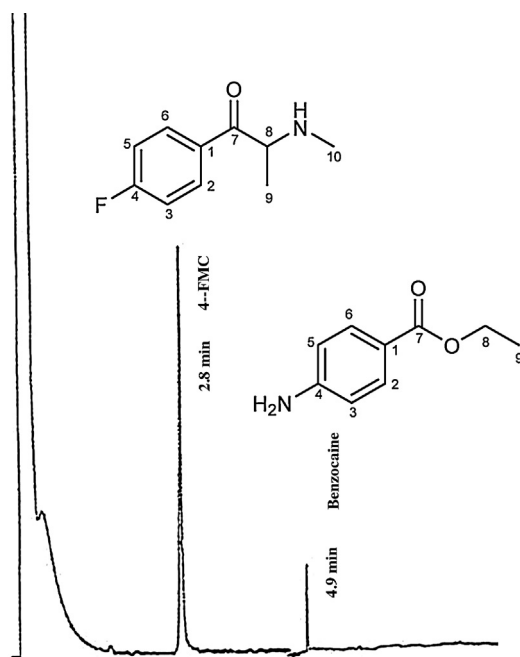


Fig. 4. GC-FID of flephedrone samples impure with benzocaine (showing ring numbering systems).

discrimination between simple drug mixtures has been achieved using a deep ultraviolet-visible (DUV-vis) reflected optical fibre sensor [24]. Both techniques can detect and possibly quantify known drugs for which standard spectra are available, but are not suitable for the analysis of new drugs.

4. Conclusions

The combination of ^1H , ^{13}C , ^{15}N , and ^{19}F NMR spectroscopy was used to identify and decipher between flephedrone regioisomers and other cathinones such as mephedrone. We have separated flephedrone and benzocaine with base-line resolution by GC-FID and quantified the amount of benzocaine adulterant in the four (nightclub) flephedrone samples by GC, the ^1H NMR integration was in broad agreement. These data show that this now illegal cathinone-derived stimulant (a high) is now being cut by 5–12% with benzocaine, so drug users cannot be certain of the purity of the drug they are taking. Benzocaine as an adulterant can cause serious health problems, potentially more harmful than flephedrone. Therefore, there are risks from the pharmaceutically active cutting agents themselves.

Acknowledgements

We thank the Government of Saudi Arabia for a Scholarship (to MA) and the Avon and Somerset Constabulary for the collection and provision of the amnesty bin. We also thank Dr R. P. Archer, States Analyst's Laboratory, Guernsey, for helpful discussion of NMR spectroscopic data.

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Analysis of a Bristol amnesty bin as an indicator of current drugs trends

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Abstract - An amnesty bin was offered by the police at a nightclub in Bristol. It was found to contain different numbers of various illegal drug samples. These were provided as: powders or crystals in folded paper or plastic bags, tablets, capsules, or as “rocks” or “bombs” (a twisted cigarette paper). Legal highs, medicinal and herbal (cannabis) drugs were detected therein. ^1H and ^{13}C NMR spectroscopy was used as the main analytical technique supported by electrospray ionisation-mass spectrometry (ESI-MS). 2D NMR (^{15}N , H_2BC , HMBC, DEPT) analysis was run in some cases to provide additional analytical data. The samples contained drugs from different classes, class A (cocaine, crack cocaine, ecstasy), class B (mephedrone, flephedrone, cannabis), and class C (ketamine).

INTRODUCTION

In the UK, drugs users represent 8.9% of the population [1], and the widespread use of drugs is estimated to cost society £15.4 bn annually [2].

MATERIALS AND METHODS

The Police made available ~200 tablet and powder samples from an amnesty bin provided in a Bristol (UK) nightclub (2013) (in addition to ~20 herbal samples that were not analysed further). Each tablet and powder sample was weighed, labelled, analysed, and identified without any initial purification. Samples (20-50 mg) were dissolved in deuteriated solvent (0.6 mL) for Nuclear Magnetic Resonance (NMR) spectroscopy, as well as analysed by Fourier-transform infrared spectroscopy (FT-IR) and ESI-MS analysis. All measurements were run at 20-25 °C on: a Bruker 400 MHz NMR Spectrometer, a Perkin Elmer-Spectrum 65-FT-IR Spectrometer, and a Bruker Daltonics microTOF.

RESULTS AND DISCUSSION

A total of seven different classes of drugs were found in the amnesty bin. A crystalline form of ecstasy (MDMA) was clearly the dominant drug 68% (129 samples) (see Fig. 1) alongside 2% (4) MDMA freebase. The second most common drug was ketamine (freebase) 10% (19) followed by: mephedrone (salt) 7% (13); benzocaine 6% (11); cocaine (salt) 3% (6); flephedrone (salt) 2% (4); one sample of cocaine freebase (crack) and one of Benzo Fury provided for “chemical research”. Of the mephedrone samples, 9 out of 13 were cut with monosodium glutamate (MSG) either as an easily accessible compound or due to it being similar in appearance. All the flephedrone samples were diluted (cut) with benzocaine, a topical anaesthetic.

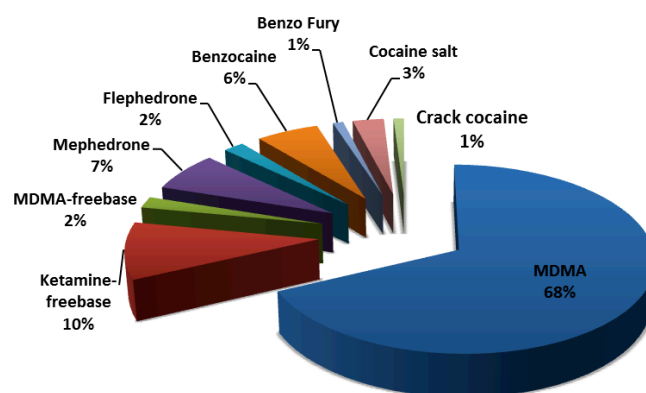


Fig. 1. Substances in an amnesty bin from a Bristol nightclub (2013).

A few samples were found to contain such an adulterant or cutting agent alone, e.g. benzocaine, creatine, or sugar. Benzocaine can mimic the numbing effect of cocaine and might possibly be sold as cocaine.

CONCLUSIONS

Amnesty bin analysis can provide potential information about currently abused drugs and possibly about such drug availability. One aim of this study is therefore to elicit any trends in such drug abuse, and then to corroborate them with regional data to provide better intelligence for police drug control. Drug formulation may determine the route of administration e.g. a freebase is likely to be smoked, whilst the salt form is mostly administered as an injection or drunk if mixed with alcohol. Despite emerging concerns over new psychoactive substances (NPS), class A drugs are still available. These analytical results show the application of NMR spectroscopy in drug detection with no need for sample preparation, reference material, or internal standard.

ACKNOWLEDGMENTS

We thank the Government of Saudi Arabia for a Scholarship (to MA) and the Avon and Somerset Constabulary for the collection and provision of the amnesty bin.

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Forensic analysis of a Glastonbury amnesty bin: Evaluation of compact spectrometer techniques compared to NMR

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Forensic analysis of amnesty bins provides reliable and quantitative data on identity and purity of drug substances. Identification of cutting agents and their ratios can help link directly to a manufacturer or trafficking network. ATR-FTIR equipped with the TICTAC drug identification database was evaluated in this study and compared to NMR to provide a rapid, precise test for identifying substances and their impurities in an amnesty bin. The Glastonbury music festival (2013) amnesty bin samples were obtained from the Drug Expert Action Team (DEAT), Avon and Somerset Constabulary, UK. ATR-FTIR spectroscopic analysis was performed on a Bruker FT-IR spectrometer (ALPHA Bruker Optics, Billerica, MA, USA). ^1H NMR data were collected on a Bruker 500 MHz NMR spectrometer and NMReady-60 PRO (Nanalysis Corp., Canada).

ATR-IR was successfully used to identify a wide variety of illicit drug samples, e.g. ketamine (22), mephedrone (33), flephedrone (4), cocaine (13), heroin (10), MDMA (76), methylone (1), and popper (14). Both ATR-IR and NMR discriminated between mephedrone and flephedrone, but benzocaine as a cutting agent was not recognized by ATR-IR. The possible diversion of street ketamine samples from legal sources was investigated by detecting the low levels of preservatives, down to 5 $\mu\text{g/mL}$, present in pharmaceutical formulations using ^1H NMR when ATR-IR could not detect such concentrations of these preservatives. Although ATR-IR provides a quick non-destructive method to identify illicit drugs in seized samples, NMR provides rapid and quantitative information on drugs, preservatives, cutting agents, and impurities.

Biography

Majdah Alotaibi is an Assistant Lecturer at the University of Tabuk, Saudi Arabia and she is in the final year of her PhD at the University of Bath, UK. Her project is focussed on “impurity profiling of illicit drugs” using different techniques, e.g. HPLC, NMR, EA-IRMS, LC-MS/MS, GC-MS and Polarography. She has 3 years experience in the quantitative analysis of illicit drugs.

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Session name number: Forensic Chemistry

Category: (Oral presentation)

To determine the geographical origin of street ketamine samples by using ^{13}C and ^{15}N stable isotope ratio analysis

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Abstract - The anaesthetic ketamine is abused for its dissociative effects resulting in it being a controlled substance. There is a need to determine the sources of the abused ketamine, in particular whether it is being diverted from legitimate veterinary and clinical supplies or manufactured abroad and imported illegally. Element analysis by isotopic ratio was employed to help answer this question. Therefore, the isotopic ratio values of 11 street samples were compared with the available ketamine pharmaceutical products, VetalarTMV and Ketalar[®], as well as an authentic ketamine HCl sample purchased from Sigma Aldrich.

MATERIALS AND APPARATUS

Eleven different ketamine samples obtained from different sources were subjected to isotopic ratio analysis, EA-IRMS analysis. VetalarTMV and Ketalar[®] were purchased as ketamine hydrochloride injections, ketamine HCl was purchased from Sigma-Aldrich. All % elemental data are traceable to The National Institute of Standards and Technology (NIST) primary references, with ^{15}N calibrated to reference IAEA-N1 ammonium sulfate and ^{13}C calibrated to reference IAEA-CH-3 cellulose.

RESULTS AND DISCUSSION

We applied the EA-IRMS technique to obtain evidence for any of the seized and amnesty bin ketamine samples we are investigating having been diverted from hospital or veterinary supplies. The $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ values of these samples were therefore compared with the data from ketamine obtained from known geographical sources, specifically VetalarTMV (Germany), Ketalar[®] (Ireland), and authentic ketamine HCl (India). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of samples of interest were determined by EA-IRMS, avoiding any extraction step to nullify potential isotopic fractionation from the solvent.

Based on the $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ (parts per thousand) values, 8 of the 11 samples can be grouped into three sets: I (seized samples), II (Bristol night club amnesty bin), and III (Glastonbury 2013 amnesty bin). The $\delta^{15}\text{N}$ ‰ isotopic ratios of the 11 samples varied between 4.175 and 13.975‰. These results show that the seized ketamine samples had almost identical values of $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ and therefore might be from a single source. Similarly, all the Bristol night club samples could be grouped together. The VetalarTMV and Ketalar[®] samples had different $\delta^{15}\text{N}$ ‰, but very close $\delta^{13}\text{C}$ ‰ values which did not match any of the illicit samples. None of the illicit samples had an isotopic ratio fingerprint comparable to the purchased ketamine (Indian). Three samples from the Glastonbury 2013 amnesty bin appeared to have distinctive profiles.

CONCLUSIONS

This study shows the feasibility of using $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ values to link or discriminate between various ketamine samples. None of the illicit samples had isotopic ratio fingerprints equivalent (or even similar) to the VetalarTMV and Ketalar[®] samples, suggesting they had not been diverted from veterinary or hospital supplies, nor purchased from Sigma-Aldrich. Therefore, they are the products of illegal synthesis.

Biography

Majdah Alotaibi is an Assistant Lecturer at the University of Tabuk, Saudi Arabia and she is in the final year of her PhD at the University of Bath, UK. Her project is focussed on “impurity profiling of illicit drugs” using different techniques, HPLC, NMR, LC-MS, ESI-MS, and GC-MS.

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Session name number: Forensic Chemistry

Category: (Oral presentation)

Profiling seized samples of mephedrone adulterated with creatine

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AIMS

A profile comparison method for the identification of impurities found in seized mephedrone (a cathinone) is reported. Creatine monohydrate was detected in these samples as an adulterant in high molar ratio creatine: mephedrone, 1.6-2.2:1 (^1H NMR integration). Samples were centrifuged in methanol and submitted to full spectroscopic characterization (1- and 2D-NMR, FT-IR, ESI-MS). Possible links between samples, based on their origins through their impurities are briefly discussed.

INTRODUCTION

With certain drug combinations comes a high risk of death. An accidental death was reported due to a combination of mephedrone (4-MMC, Fig. 1) and heroin [1]. Mephedrone has normally been supplied "on the street" in pure form, this work was to determine if this was still the case.

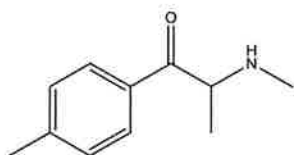


Fig. 1. Mephedrone (4-MMC)

MATERIALS AND METHODS

Three seized mephedrone samples (1, 2, 3) were obtained from the Drug Expert Action Team (DEAT), Avon and Somerset Constabulary, UK. Creatinine anhydrous, creatine monohydrate, and all other reagents used in this work were purchased commercially (Sigma-Aldrich, UK). All measurements were run at ambient temperatures on: a Bruker 500 MHz NMR (^1H and ^{13}C), a Perkin Elmer 65-FT-IR (KBr disc) Spectrometer, and a Bruker Daltonics "microTOF" (ESI-MS).

RESULTS AND DISCUSSION

Mephedrone in each of these seized street samples is present as its hydrochloride salt (4-MMC HCl). This is readily soluble in water, in contrast to its free base. The isolation of the active component (4-MMC) was achieved by dissolving the salt in methanol to separate it from the impurity, dividing into mephedrone and the adulterant. Spectroscopic profiles ^{13}C NMR (Fig. 2), FT-IR, and ESI-MS were determined in order to search for any link between the seized samples. The data show that all three samples contain the same impurity, but in different molar ratios (1.6, 2.0, and 2.2:1, indicative of supply from different batches. ^{13}C NMR (D_2O): δ 15, 20, 31, 36, 53, 59, 128, 129, 130, 147, 157, 174, 198 ppm [2]; IR: 3411 (NH), 2965 (PhH), 2743 (CH_3), 1688 ($\text{C}=\text{O}$), 1607 ($\text{C}=\text{C}$) cm^{-1} ; $[\text{M}+\text{H}]^+ \text{C}_{11}\text{H}_{16}\text{NO}$ requires 178.1226, found 178.1265; $[\text{M}+\text{Na}]^+ \text{C}_{11}\text{H}_{15}\text{NONa}$ requires 200.1051, found 200.1063.

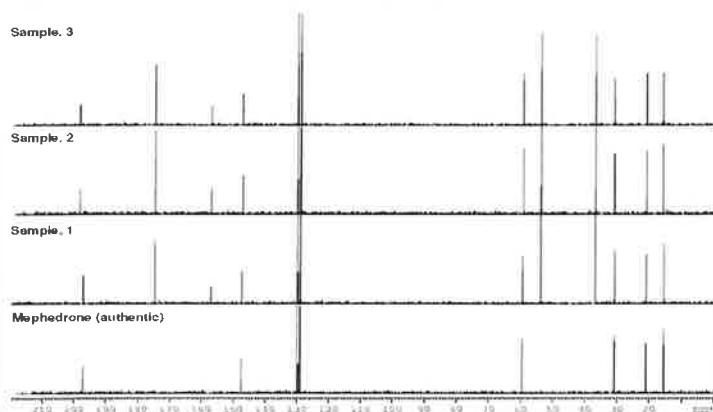


Fig. 2. ^{13}C NMR spectra (D_2O) of mephedrone (Aldrich) and samples 1, 2, 3

^{13}C NMR, FT-IR, and ESI-MS data of the impurity are identical with those of authentic creatine monohydrate. ^{13}C NMR (D_2O): δ 36, 53, 157, 174 ppm (Fig. 3). IR (KBr): 3340 (br OH), 3092 (br OH), 2786 (CH_3), 1698 ($\text{C}=\text{O}$), 1614 ($\text{C}=\text{N}-\text{H}$) cm^{-1} . ESI-MS spectra show creatine $[\text{M}+\text{H}]^+ \text{C}_4\text{H}_9\text{N}_3\text{O}_2$ requires 132.0767, found 132.0781; $[\text{M}+\text{Na}]^+ \text{C}_4\text{H}_9\text{N}_3\text{O}_2\text{Na}$ requires 154.0587, found 154.0597; $[\text{M}-\text{H}]^- \text{C}_4\text{H}_8\text{N}_3\text{O}_2$ requires 130.0622, found 130.0635.

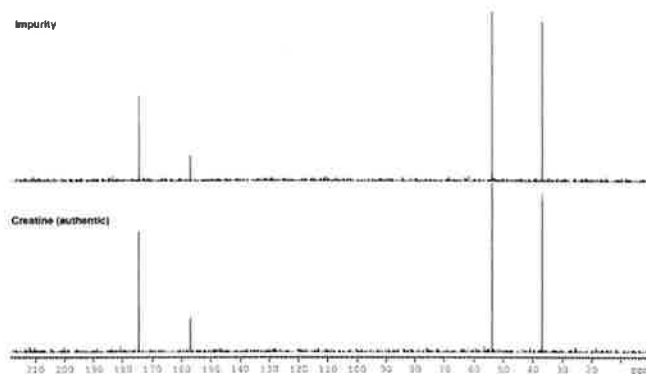


Fig. 3. ^{13}C NMR spectra (D_2O) of authentic creatine monohydrate and of creatine monohydrate, the key impurity



Fig. 4. X-ray structure of creatine

CONCLUSIONS

Chemical profiling of the creatine monohydrate cutting agent determined in this approach links samples to batches and maybe to the source of supply. This may therefore help in identifying manufacturing or trafficking organizations.

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Fast analytical methods to profile three seized ketamine samples

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AIMS

Full spectroscopic profiling (^1H NMR, ^{13}C NMR, FT-IR, UV, ESI-MS, and $[\alpha]_D^{20}$) of seized samples of ketamine were carried out and compared demonstrating that each of the three samples is of high purity (>98.7%).

INTRODUCTION

Ketamine BP is abused for its dissociative effects. In the last few years there has been a dramatic increase in the sale of "legal highs" [1] and (illegally) of prescription only medicines. For evidential and intelligence purposes, drug profiling studies can be used to link between different seizures [2]. The presence or absence of specific organic impurities in synthetic drugs can be valuable tools to link between different seizures from the same source [3].

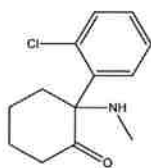


Fig. 1. (±)-Ketamine

MATERIALS AND METHODS

Materials

Three different seized ketamine (KT) samples (KT-1, 2, and 3) were obtained from the Drug Expert Action Team (DEAT), Avon and Somerset Constabulary, UK and analyzed as received. All other chemicals and reagents were purchased from Sigma-Aldrich, UK.

Apparatus

Spectroscopic measurements were recorded on a Perkin Elmer 65-FT-IR Spectrometer, a Bruker NMR (400 MHz), a Bruker Daltonics "microTOF" electrospray ionization mass spectrometer (ESI-MS), and optical rotation values were determined on an ADP-220 polarimeter (Bellingham and Stanley, Tunbridge Wells, UK).

RESULTS AND DISCUSSION

An X-ray crystal structure was obtained and shows a racemic mixture of the title compound (Fig. 2). Equal chemical structures for samples (KT-1, 2, 3) were confirmed by ^1H and ^{13}C NMR spectroscopy (Fig. 3a); full spectral analysis using both HMQC and HMBC methods allowed unambiguous assignment of all ^1H and ^{13}C resonances and indicated that each sample was pure ketamine HCl. The comparison between samples (KT-1, 2, 3) using FT-IR is shown in Fig. 3b together with the subtraction spectrum, confirming their equivalence. ^{13}C NMR (CDCl_3): δ 21.5, 28.1, 29.4, 38.5, 40.2, 72.5, 128.4, 128.5, 131.6, 131.8, 131.9, 135.1, 205.5 ppm; IR (KBr disc) 3423 (N-H), 1722 (C=O), 774 (C-Cl or 4 x Ph-H) cm^{-1} ; UV (MeOH) λ_{max} = 269 and 276 nm; ESI-MS (positive ion mode) calculated for $[\text{M}+\text{H}]^+$ $\text{C}_{13}\text{H}_{17}^{35}\text{ClNO}$ requires 238.0993, found 238.0982; $\text{C}_{12}^{13}\text{CH}_{17}^{35}\text{ClNO}$ requires 239.1027, found 239.1034; and $\text{C}_{13}\text{H}_{17}^{37}\text{ClNO}$ requires 240.0964, found 240.0966 (ratio 3:1); $[\alpha]_D^{20}$ = 0 (c = 1 mg/mL, MeOH). Equal amounts of both enantiomers are present in each of the seized ketamine hydrochloride samples.

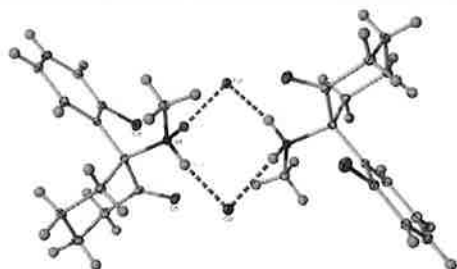


Fig. 2. X-ray structure of (±)-ketamine

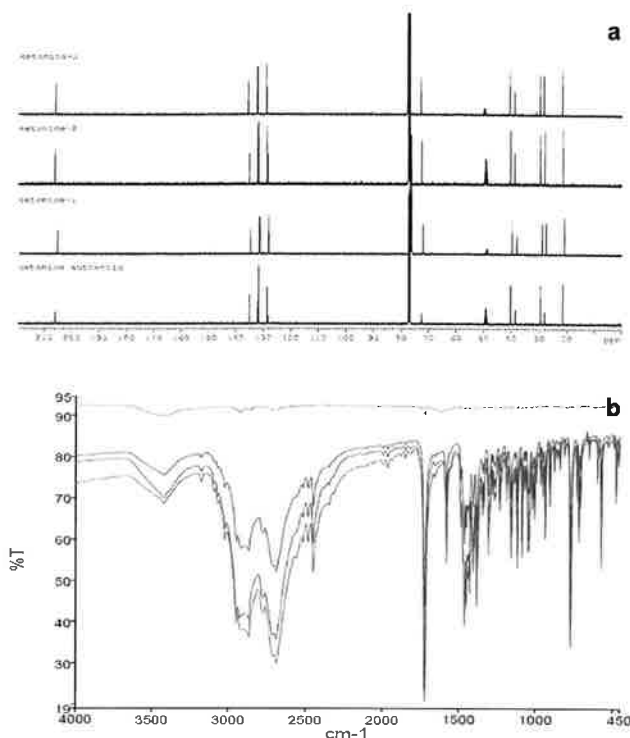


Fig. 3. (a) ^{13}C NMR (CDCl_3) and (b) FT-IR spectra (KBr) for KT-1 (—), 2 (—), 3 (—), and the subtraction spectrum (---)

CONCLUSIONS

The results confirm that these three separate samples of ketamine, seized by the police, are equivalent and of high purity (>98.7%). The samples have not been adulterated (cut) with other materials including inorganic (CDCl_3 insoluble) material. Whilst these three ketamine samples are all racemic, this is mostly how ketamine BP is supplied. However, pharmacokinetic properties of (R)-(S)-ketamine have been proved to be significantly different. They have been reported to have different metabolic profiles and receptor binding affinities, with (S)-ketamine being a more potent analgesic agent [4,5]. The results show the potential of this method to discriminate between ketamine samples and any adulterants.

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Chemical characterisation of seized flephedrone by ^1H , ^{13}C , and ^{19}F NMR spectroscopy

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Abstract – The identification of fluorine-containing drugs and their metabolites can potentially be achieved using NMR spectroscopic analysis. Pharmaceutical analysis of flephedrone (4-fluoromethcathinone, 4-FMC) included ^1H , ^{13}C , ^{19}F , ^{15}N HMBC and ^{15}N HSQC spectroscopy and electrospray ionisation-mass spectrometry (ESI-MS). Analysis of four 4-FMC samples (from a nightclub in 2013) showed that they all contained benzocaine as a cutting agent, used in different ratios from 7-13%.

INTRODUCTION

Flephedrone (4-fluoromethcathinone, 4-FMC) (Fig. 1) was first synthesized in 1952, but surprisingly there is a paucity of published data for this compound. Compared with cathinone, the C-F bond on an aromatic ring shows a specific polarity effect that might influence receptor binding and therefore influence the biological activity.

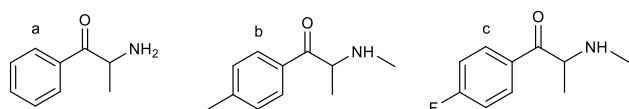


Fig. 1. Cathinone (a), mephedrone (b), and flephedrone (c).

MATERIALS AND METHODS

Four flephedrone samples were identified in an amnesty bin from a nightclub in Bristol (UK) in 2013. The samples were analysed, without any initial purification, at 20-25 °C. All reagents were purchased from Sigma-Aldrich, UK. Spectroscopic measurements were recorded on a Bruker 500 MHz NMR Spectrometer, a Perkin Elmer-Spectrum 65-FT-IR Spectrometer, and a Bruker Daltonics micrOTOF.

RESULTS AND DISCUSSION

A spectroscopic dataset (NMR, FT-IR, MS) was acquired for the hydrochloride salt samples. The structures were confirmed by comparing the spectroscopic data with the literature values [1]. ^1H NMR (D_2O) δ 8.09-8.05 (2H, m, ArH), 7.33-7.29 (2H, m, ArH), 5.09 (1H, q, J 7 Hz, α -CH), 2.81 (3H, s, N- CH_3), 1.60 (3H, d, J 7 Hz, α - CH_3); ^{13}C NMR (D_2O) δ 196 (C=O), 167 (C-F, d, J 254 Hz), 132 (CH_{arom} , d, J 10 Hz), 129 (Cq, d, J 3 Hz), 116 (CH_{arom} , d, J 22 Hz), 59 (CH), 31 (N- CH_3), 15 (α - CH_3) ppm; $[\text{M}+\text{H}]^+ \text{C}_{10}\text{H}_{13}\text{FNO}$ requires 182.0981, found 182.0988. Spectral data are compared with mephedrone (4-methylcathinone), a closely related cathinone derivative, and the 4-FMC was also shown to be impure (i.e. 7, 9, 10, and 13%) with benzocaine [2] using ^1H and ^{13}C NMR data.

¹⁹F NMR spectroscopy

The ^1H NMR spectrum of 4-FMC shows high similarity to cathinone and mephedrone (Fig. 1) and this can lead to mis-labelling. ^{19}F NMR spectroscopy proves the presence and position of a fluorine atom in such samples. Comparing with the literature data [1], the ^{19}F NMR chemical shift values for the 4-FMC samples show *para*-substitution: ^{19}F NMR -102.1 ppm (tt, $^3J_{\text{HF}}$ 10 Hz, $^4J_{\text{HF}}$ 5 Hz) (Fig. 2).

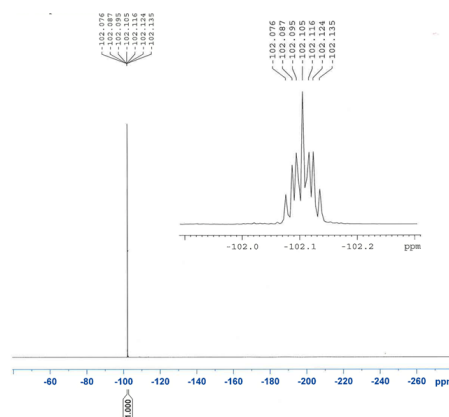


Fig. 2. ^{19}F NMR spectrum of flephedrone (in the presence of benzocaine).

CONCLUSIONS

¹⁹F NMR is a useful characteristic signal to decipher between flephedrone and other cathinones by providing a unique peak at -102 ppm for *para*-cathinone substituted with fluorine. Benzocaine is a common cutting agent that has been used in cutting cocaine and other drugs, which might link such adulterated flephedrone to other commonly cut drugs [2]. Finally, any harm associated with benzocaine as an adulterant can cause a serious health problem, more harmful than the illicit drug flephedrone itself.

ACKNOWLEDGEMENTS

We thank the Government of Saudi Arabia for a Scholarship (to MA) and the Avon and Somerset Constabulary for the collection and provision of the amnesty bin.

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Carbon and nitrogen stable isotope ratio analysis of seized and amnesty bin ketamine samples to determine their geographical origin

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Abstract - The anaesthetic ketamine is abused for its dissociative effects resulting in it being a controlled substance. There is a need to determine the sources of the abused ketamine, in particular whether it is being diverted from legitimate veterinary and clinical supplies or manufactured abroad and imported illegally. Isotopic ratio analysis was employed to help answer this question. The isotopic ratio values were compared with commercially available pharmaceutical products of ketamine, VetalarTMV and Ketalar[®], as well as an authentic ketamine HCl sample from Sigma Aldrich.

INTRODUCTION

Reports from UK police services suggest increasing ketamine (KT) abuse in many UK regions [1]. The majority of illicit KT is pure, not adulterated, suggesting possible diversion from hospital or veterinary sources rather than synthesis [2]. Isotopic ratio analysis has been utilised to establish the geographical origin of various substances, including drugs of abuse and their precursors.

MATERIALS AND APPARATUS

Eleven different ketamine (KT) samples from different sources (Table 1) were subjected to EA-IRMS analysis. VetalarTMV and Ketalar[®] were purchased as ketamine hydrochloride injections. Authentic ketamine HCl was purchased from Sigma Aldrich. All % element data are traceable to The National Institute of Standards and Technology (NIST) primary references, ¹⁵N calibrated to IAEA-N1 ammonium sulfate and ¹³C calibrated to reference IAEA-CH-3 cellulose. ¹H NMR data were collected on a Bruker (500 MHz NMR) spectrometer. Mass spectrometry was recorded on a Bruker Daltonics “microTOF” electrospray ionization mass spectrometer (ESI-MS).

Table 1. Identification of KT samples by origin

Sample	Country of origin	Supplier
Vetalar TM V	Germany	Zoetis UK Limited
Ketalar [®]	Ireland	Pfizer Ltd, Kent, UK
Ketamine HCl	India	Sigma Aldrich (authentic)
GB2-1, GB3-2, GB4-1, GA7, GA11, GB14-11	Glastonbury music festival amnesty bin, (2013)	Avon and Somerset Constabulary, UK
AH9, AS5, AV1	A night club in Bristol, UK (2013)	Avon and Somerset Constabulary, UK
K2, K3	Seized samples, Avon and Somerset Constabulary	Drug Expert Action Team (DEAT), UK

RESULTS AND DISCUSSION

We applied the EA-IRMS technique to provide evidence for any of the seized and amnesty bin KT samples having been diverted from hospital or veterinary supplies. $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ values of these samples were compared with those from known sources, specifically VetalarTMV, Ketalar[®], and authentic ketamine HCl.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of samples of interest were determined by EA-IRMS, avoiding any extraction step to nullify potential isotopic fractionation from the solvent. Approximately 10 mg of the ketamine samples were accurately weighed and submitted to the EA-IRMS in a tin capsule.

Based on the $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ (parts per thousand) values (Fig. 1), 8 of the samples could be grouped into three sets: I (seized samples), II (Bristol night club), and III (Glastonbury). The $\delta^{15}\text{N}$ ‰ isotopic ratios of the 11 samples varied between 4.175 and 13.975 ‰. These results showed that the seized ketamine samples (Group I) had almost identical values of $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ and therefore might be from a single source. Similarly, all Bristol night club samples could be grouped together. The VetalarTMV and Ketalar[®] samples had different $\delta^{15}\text{N}$ ‰ but very close $\delta^{13}\text{C}$ ‰ values and did not match any of the illicit samples. None of the illicit samples had an isotopic ratio fingerprint comparable to the authentic ketamine (Indian). Three samples from the Glastonbury amnesty bin appeared to have distinctive profiles.

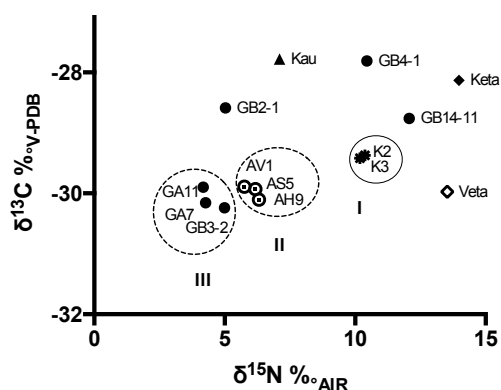


Fig. 1. $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ values for the seized KT samples (+), Glastonbury amnesty bin (•), Bristol night club (o), VetalarTMV (◊), Ketalar[®] (♦), and Ketamine HCl (▲), values are reported as duplicates.

CONCLUSIONS

This study shows the feasibility of using $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ values to link or discriminate between various ketamine samples. None of the illicit samples had isotopic ratio fingerprints equivalent (or even similar) to the VetalarTMV and Ketalar[®] samples, suggesting they had not been diverted from veterinary or hospital supplies.

ACKNOWLEDGMENTS

We thank the Government of Saudi Arabia for a fully-funded Scholarship (to MA).

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Impurity profiling of seized and amnesty bin ketamine samples to determine if any diversion from hospital or veterinary sources to drug users in the UK

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Abstract – Anaesthetic ketamine B.P. is now also known as a drug of abuse. Pharmaceutical products VetalarTMV and Ketalar[®] were analysed by spectroscopic profiling (¹H and ¹³C NMR, [α]_D²⁰, ESI-MS and UPLC/MS/MS) together with many seized and amnesty bin samples of ketamine. It is likely that the drug being abused is synthetic rather than diverted B.P. samples due to the absence of any preservative.

INTRODUCTION

Misuse of ketamine appeared in the 1960s [1], but this was not particularly common in Europe until ketamine was used as an adulterant in ecstasy tablets in the 1990s [2]. The majority of ketamine is pure and not adulterated, but is this diverted from pharmaceutical companies, hospital or veterinary clinics rather than of synthetic origin, even on a large scale [3]? Based on a most recent update (02.04.15), the Veterinary Medicine Directorate (VMD) product information database, authorised in the UK, ketamine products are available with any of these excipients: benzethonium chloride, disodium edetate or chlorocresol in solution for injection. However, hospital versions are available with/or without benzethonium chloride as a preservative. The presence of specific organic impurities, including excipients in medicines, can be a valuable aid to identify and to discriminate between possible sources.

MATERIALS AND METHODS

Three different seized ketamine (KT) samples (KT-1, 2, and 3) were obtained from the Drug Expert Action Team (DEAT), Avon and Somerset Constabulary, UK, 39 samples were from the Glastonbury music festival (2014) and from a night club in Bristol, UK (2013). Ketamine Hydrochloride Injections, VetalarTMV and Ketalar[®] (100 mg/mL) with benzethonium chloride as preservative, 0.01% and 1% w/v respectively, were from Pfizer Ltd (Kent, UK). Reagents and solvents were purchased from Sigma-Aldrich, UK.

Chromatographic separation was performed on an Ultra High Performance LC (UPLC/MS/MS) system (Thermo Scientific, Milford, MA, USA); a UPLC method developed to detect both ketamine and benzethonium chloride. NMR data were collected on a Bruker (500 MHz) spectrometer. Optical rotation data were determined on an ADP-220 polarimeter (Bellingham and Stanley, Tunbridge Wells, UK).

RESULTS AND DISCUSSION

A water suppression technique PURGE (Presaturation Utilizing Relaxation Gradients and Echoes) [4] was used to detect ketamine and any excipients in the injectable solutions of VetalarTMV and Ketalar[®]. ¹³C satellites peaks were used as the internal standard to calculate sample purity.

Many NMR scans were required to achieve good signal/noise for benzethonium chloride at 0.01% w/v. Ketamine ¹³C NMR (D₂O): δ 21.5, 28.1, 29.4, 38.5, 40.2, 72.5, 128.4, 128.5, 131.6, 131.8, 131.9, 135.1, 205.5 ppm.

[α]_D²⁰ = 0 (c = 1 mg/mL, MeOH). ESI-MS (positive ion mode) calculated for [M+H]⁺ C₁₃H₁₇³⁵ClNO requires 238.0993, found 238.0982. Positive ion ESI-MS also showed a molecular ion at m/z 412.3403 which matched the m/z value of authentic benzethonium chloride.

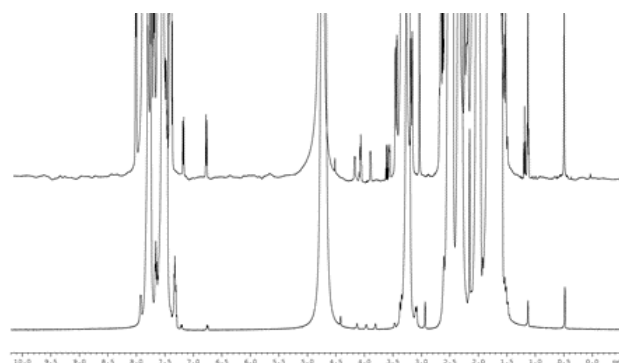


Fig. 1. ¹H NMR of VetalarTMV in D₂O (lower) and ketamine spiked with benzethonium chloride (upper).

CONCLUSIONS

Equal amounts of both enantiomers are present in each of the seized and amnesty bin ketamine hydrochloride samples. Based on our results, there is no evidence yet that ketamine is diverted from hospital or veterinary sources, even though the ketamine used in the UK is a racemic mixture of enantiomers. There is potential that imported versions could be (only) *S*-ketamine, e.g. as used in Australia, which is about twice as potent as *R*-ketamine, and so with a concomitantly higher risk of overdose.

ACKNOWLEDGMENTS

We thank the Government of Saudi Arabia for a fully-funded Scholarship (to MA).

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Chemical profiling of MDMA samples and their impurities within a clinical study on psychoactive effects of MDMA on users

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Abstract - Seventeen samples, provided by the study participants, were not of pharmaceutical grade and while they were believed to contain MDMA (ecstasy) this needed to be confirmed authoritatively as part of a clinical study into the unique sociotropic effects of MDMA. In order to identify the components of the samples, in particular, to determine if other psychoactive substances were present, the crystalline samples were subjected to LC/MS/MS and detailed NMR analysis.

INTRODUCTION

There has been a revival of interest in the use of psychedelic and stimulant drugs as therapeutic agents with studies suggesting that some compounds (for example psilocybin) can induce positive, long-lasting personality changes akin to those mediated by prolonged and intensive psychotherapy. 3,4-Methylenedioxy-N-methylamphetamine (MDMA, ecstasy) has a unique and complex pharmacology, acting on the monoamine, oxytocin and vasopressin systems [1]. Use of MDMA has been linked to feelings of enhanced interpersonal relatedness, or a sense of connection to others, activities that have contributed to it being evaluated in a number of small-scale clinical trials as an adjunct to psychotherapy. A recent study examined whether ecstasy (and therefore, presumably, MDMA) use caused subjectively positive or therapeutically relevant effects in recreational users [2]. While the findings were supportive of a role for ecstasy/MDMA and its proposed use as an adjunct to psychotherapy, no chemical analysis was performed on the ecstasy samples. Thus, MDMA dose/purity, or even whether the samples contained any MDMA, was unknown. This current study therefore aims to determine the composition of each ecstasy sample used in a similar, recently completed, clinical study.

REAGENTS AND STANDARDS

Methoxyphenamine hydrochloride and formic acid (98-100%) were purchased from Sigma Aldrich. A pure (\approx 99%) sample of MDMA was used as reference material. Deuteriated (d_4) methanol with 0.05% TMS as internal standard was used for NMR analysis and quantification. All other solvents were of HPLC grade.

APPARATUS

All ^1H NMR data were collected on a Bruker (500 MHz NMR) spectrometer; mass spectrometric data were recorded on a Bruker Daltonics "microTOF" electrospray ionization mass spectrometer (ESI-MS). Chromatographic separation was performed on an Ultra High Performance LC (UPLC/MS/MS) system (Thermo Scientific, Milford, MA, USA).

RESULTS AND DISCUSSION

UPLC/MS/MS and NMR investigation showed that 16 out of 17 samples contained MDMA. Approximately half of these samples were highly pure with MDMA content $>99\%$ (Table 1). Of those that had been cut with other substances, purity ranged from $<50\%$ to 83% MDMA. Four samples contained sugar (sucrose and/or glucose) alone or in the presence of another substance (in one case, this was a psychoactive substance, mephedrone), one sample was cut with cocaine (1:1) and the remaining sample (as provided and taken by the participant) had no MDMA present.

Table 1. ^1H NMR (500 MHz) of illicit drug samples (CD_3OD 0.05%TMS).

Participant no.	Psychoactive Substances	Impurity	MDMA Purity (%)
1	MDMA	–	>99
2, 3	MDMA	–	>99
4	MDMA	–	>99
5	MDMA	–	>99
6, 7, 11	MDMA	–	>99
8, 9	MDMA	–	>99
10	MDMA	–	>99
12	MDMA	glucose	83%
13, 14	MDMA	–	>99
17, 18	MDMA	–	>99
19	unknown	unknown	
20	MDMA	–	>99
21	MDMA	glucose	77%
22	MDMA, mephedrone (12%)	sucrose	77%
23, 24	MDMA	sucrose + unknown	$<50\%$
25	MDMA	unknown insoluble	soluble materials $>99\%$
27	MDMA, cocaine (50%)	–	50%

*In sample 25 a small amount of insoluble material was found

CONCLUSIONS

These results will be invaluable in helping to interpret the findings of the associated clinical study. They confirm the importance of verifying the chemical composition of drug samples used by recreational users as MDMA content of the ecstasy samples employed in the current study varied from 0 to $\sim 100\%$. The presence of other psychoactive substances such as cocaine and mephedrone further complicates interpretation of the clinical studies.

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Impurity profiling of MDMA tablets in the UK:

A combination of benchtop FT-IR equipped with TICTAC database and ^1H NMR

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Abstract – MDMA tablets (75) were received from the police from a Bristol night club (2013) and Glastonbury amnesty bins (2013). All the tablets were identified from the TICTAC library using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) followed by ^1H NMR spectroscopy and confirmed by Electrospray Ionisation Mass Spectrometry (ESI-MS). All 75 tablets were correctly identified using benchtop ATR-FTIR as containing MDMA, but the impurities were not always accurately identified. An advantage of NMR over FT-IR is that almost all the soluble contents of the MDMA tablets were accurately identified by NMR.

INTRODUCTION

MDMA is very popular as party drugs in Europe. These drugs commonly available as tablets [1] structure (Fig. 1). According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), the composition of the MDMA tablets is changing all the time in Europe [2].

MATERIALS AND METHODS

The Glastonbury music festival (2013) and the Bristol night club (2013) amnesty bins samples were obtained from the Drug Expert Action Team (DEAT), Avon and Somerset Constabulary, UK. Recrystallized MDMA was prepared from pure street samples, analysed by NMR and used as a reference material. Reagents and solvents were purchased from Sigma-Aldrich, U.K. ATR-FTIR spectroscopic analysis was performed on a Bruker FT-IR spectrometer (ALPHA Bruker Optics, Billerica, MA, U.S.A.) equipped with the TICTAC drugs identification database. ^1H NMR data were collected on a Bruker 500 MHz NMR spectrometer. ESI-MS were obtained on a Bruker MS.

RESULTS AND DISCUSSION

75 MDMA tablets from DEAT composed of 19 different logos, sizes, and colours (Fig. 1). An FT-IR spectrum from a pure sample of MDMA matched well with a spectrum in the TICTAC library (Fig. 2). In the analysis of these tablets, this protocol was followed: tablets were labelled, weighed, photographed, and then ground. Part (30 mg) of the ground powder was mixed in d_4 -MeOD (0.6 mL) to extract MDMA with brief centrifugation to free from insoluble material, e.g. tablet filler or cornflour. MDMA was confirmed by ESI-MS. Another part of the ground powder was used to obtain ATR-FTIR data which were automatically matched with the TICTAC database. There was good correlation between pure MDMA tablets and the database. However, in tablets containing less than 50% MDMA, the mixture is identified incorrectly by this ATR-FTIR library-based approach, e.g. a tablet which contained MDMA, caffeine, and amphetamine (by ^1H NMR) was identified as 50% MDMA and 49.8% odol whitening.



Fig. 1. MDMA tablets detected in the Glastonbury festival (2013) and the Bristol night club (2013) amnesty bins.

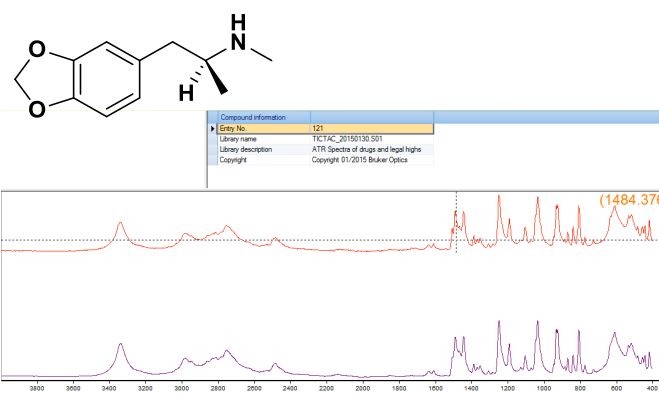


Fig. 2. FT-IR spectrum of a pure MDMA tablet (lower) with the matching MDMA (ecstasy) spectrum (upper) from the TICTAC database.

CONCLUSIONS

^1H NMR is a powerful technique to identify MDMA and soluble impurities in seized tablets. The Alpha benchtop FT-IR provides a quick characterization of drugs, e.g. MDMA, in seized materials, with the benefit of simple sample handling, but mixture analysis is challenging and not accurate by FT-IR. Impurity profiling of the contents of the seized MDMA tablets using only the ATR-FTIR technique (equipped with TICTAC) is not yet sufficiently accurate.

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